San Francisco Estuary Regional Monitoring Program for Trace Substances

Conceptual Framework and Rationale for the Exposure and Effects Pilot Study

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. BACKGROUND

In response to the revision of the RMP objectives in 1997 and the recommendations of the redesign workgroups, the RMP began development of a new component for the Status and Trends Program to monitor biological exposure and effects of contaminants in the San Francisco Estuary. The RMP is implementing this 5 year Pilot Study to develop several indicators, using resident species, that can monitor contaminant exposure and effects at several trophic levels, at different levels of biological organization (biochemical, individual, population, and community levels), and at different spatial scales (locally or regionally).

A small Workgroup was formed in 2001 to begin the planning. The objectives for 2001 were to develop management questions, review existing literature, solicit recommendations from the local scientific community (by using a survey), and come up with a preliminary plan for the Exposure and Effects Pilot Study (EEPS). In 2002 the EEPS began, with a program of study developed under the guidance of the Exposure and Effects Workgroup (EEWG). EEWG meetings and field studies began in 2002.

A goal of this pilot study is to arrive at a 'tool box' of locally tested and validated, biological exposure and effects indicators that can be used to monitor the Estuary at different temporal and spatial scales. In the long term these indicators will help inform the Regional Board on questions related to managing and protecting beneficial uses. An effort will be made to take advantage of other studies in the Estuary in order to obtain as much ancillary information as possible (for interpretive purposes) at minimal cost.

To ensure close linkages between exposure and effects measurements, and to aid in interpretation of results, this Pilot Study includes two complementary efforts:

A) Development of relevant contaminant effects indicators for benthic community assessments and higher trophic level consumers (fish, birds, and seals).

B) Linking observed biological effects to possible pollution exposure by studying bioaccumulation and biomagnification in species at various trophic positions in the food web. Performing possible biochemical marker studies and conducting contaminant sensitivity studies for important estuarine species used in toxicity tests will augment effects studies.

II. GENERAL APPROACH

A. OVERALL OBJECTIVE AND MANAGEMENT QUESTIONS

Revised objectives for the RMP were an outcome of the 1997 Program Review, including a new objective regarding effects of contaminants in the Estuary:

> "Measure contaminant effects on selected parts of the Estuary ecosystem."

The objectives were developed in the context of a framework of focusing questions and management questions for the RMP. The RMP management questions associated with the effects objective were:

a) Which contaminants bioaccumulate in estuarine organisms to levels of concern?

b) What is the spatial and temporal extent of toxicity in the Estuary?

c) Which contaminants cause effects in the Estuary?

This objective and these management questions motivated the development of the Exposure and Effects Pilot Study. Prior to the EEPS, Management question "b" was already being addressed to a large extent by aquatic and sediment toxicity testing being performed in the Status and Trends portion of the RMP. In contrast, management questions "a" and "c" were not being addressed in a significant way. Conspicuously lacking were any studies of accumulation and effects in higher trophic levels, where effects of several contaminants of greatest concern in the Estuary (such as mercury and PCBs) would be most likely. The EEPS was designed to address these deficiencies, and to enhance assessment of toxicity in the Estuary.

Planning for the EEPS began in 2001. As part of this planning process, a more detailed set of management questions was developed by Karen Taberski and Lynn Suer of the Regional Board (Appendix 1). These management questions are specifically associated with the beneficial uses designated for the Bay in the Basin Plan.

1. Indicator Selection

General Considerations

Given the many questions posed by managers, the diversity of contaminants, and the diversity of species potentially impacted by contaminants, it was clear that a multifaceted approach to assessing toxic effects in the Estuary was necessary. Public concern for the health of Bay fish, bird, and marine mammal populations also calls for a multifaceted approach. Early planning for the EEPS articulated the goal of developing a 'tool box' of locally tested and validated, biological exposure and effects indicators that can be used to monitor the Estuary at different trophic levels, levels of biological organization (biochemical, individual, population, and community levels), and spatial scales (locally or regionally). A literature review (Lowe *et al.,* 2001) and survey of local scientists was performed to develop an initial list of potential exposure and effects indicators for consideration (Table 1). A RMP workshop on ecological indicators held in 1995 (SFEI 1997) also provided background on potential monitoring approaches.

A major challenge in implementing the EEPS has been attempting to answer the many management questions posed with a finite amount of money. The EEPS is being funded at $200,000 per year over the course of five years. The two principal approaches to meeting this challenge have been: 1) careful prioritization of potential studies, and 2) piggybacking on other existing projects and collaborating with other organizations. Prioritization has been achieved by evaluating proposed elements against a set of selection criteria (discussed further below). Several opportunities for piggybacking were identified through initial meetings of the EEWG and discussions with local researchers. In fact, most of the indicator studies to date have been implemented through piggybacking on other previously existing projects.

Selection Criteria for Each Indicator

A suite of criteria have been identified to guide selection of indicators for inclusion in the Pilot Study. A brief explanation of each criterion is provided below. A detailed description of how each indicator included in the EEPS compares against these criteria is provided in Section IV and a summary is provided in Table 4 (attached).

Relevance to management questions – Each indicator must address at least one of the three RMP management questions (listed above) relating to the RMP objective to "measure contaminant effects on selected parts of the Estuary ecosystem." In addition, each indicator should address the management questions developed by Karen Taberski and Lynn Suer that are tied to beneficial uses of the Bay (Appendix 1).

Influence on management decisions –Indicators where findings would have clear consequences for managers should receive a higher priority. Ideally, each indicator should have a clear evaluation goal that will serve as a trigger to implement a management action.

Protecting species of public concern – Some species that depend on the Bay are the focus of more public attention and concern than others. The Endangered Species Act, Marine Mammal Act, and sport related issues heighten public interest in high visibility species such as harbor seals, various bird species, and various fish species. These species are of higher public interest than species such as amphipods (although the public can understand the need to protect the food supply). This doesn't mean that the RMP should be completely restricted to high visibility species, but that this should be one of the considerations. Other criteria to consider regarding species selection are ecological significance and sensitivity, as discussed under other criteria.

Likelihood of finding ecologically significant effects – EEPS should focus on the indicators with the highest probability of exhibiting ecologically significant contaminant-induced effects. Indicator species where the combination of exposure and sensitivity translate to the greatest risk should be selected. The significance of the effect refers to linkage to effects that may be linked to population, and possibly community level impacts through effects on growth, reproduction, or survival. For example, clapper rails have been demonstrated to be relatively sensitive to methylmercury in their eggs. Concentrations have been measured and are thought to be embryotoxic. The Bay population of this endangered species is producing fewer offspring than similar species in other parts of the country. Clapper rails and mercury is therefore a case with a relatively high probability of finding an ecologically significant effect.

Potential for linkage of laboratory and field studies – A key to linking specific contaminants with effects in the field is the ability to conduct laboratory studies that complement field studies. If effects observed in specimens in the field can be duplicated with controlled exposures in the lab, a strong case can be built implicating particular contaminants. Lab studies can also provide reference points, verifying that concentrations observed in the field are high enough to pose a significant health risk. Conducting lab studies of this nature demands a capability to maintain viable life stages of the indicator species in a laboratory or controlled environment. Examples of this type of linkage include the work by Bob Spies on Bay starry flounder in the 1980s (Spies *et al.,* 1988) and the recent egg injection work performed by Gary Heinz (Heinz, 2003) in support of field studies of mercury contamination in avian eggs by Steve Schwarzbach and Terry Adelsbach (Schwarzbach *et al.,* 2003 and Schwarzbach and Adelsbach, 2003).

Availability of literature on the indicator to aid interpretation – Results of studies conducted in other ecosystems can be beneficial in important ways, including establishing thresholds for toxicological concern, linking cause and effect, and identifying new methods or endpoints to evaluate. Not having to build knowledge on an indicator entirely from local studies is a big advantage.

Temporal and spatial integration – Depending on their life histories, movements, and foraging behavior, different species are indicators of different windows in space and time. Some indicator species, such as harbor seals and double-crested cormorants, reflect regional conditions because they have relatively wide foraging ranges. Other indicators, such as benthic invertebrates, reflect local conditions. Either type of indicator can be valuable for specific applications. The temporal integration provided by the indicator is also important, and needs to be carefully considered. Longer-term integration is often desirable as it provides a better indication of average conditions. Temporal and spatial integration is especially important for the exposure indicators.

Providing information needed to decide on inclusion in the Status and Trends Program – Indicator studies included in the EEPS should have a high probability of providing a basis for deciding whether the indicator has a place in long-term monitoring.

Cost – The selected indicators should have a high yield of benefit per unit cost. Indicators that are useful and sustainable in long-term monitoring are usually simple and inexpensive. Expensive indicators become hard to justify when budgets shrink, time series can get interrupted, and the indicators can be dropped. Indicators that can be implemented through collaborations with other programs (“piggybacking”) are advantageous, yielding more information per RMP dollar.

Linkage to contaminants – The ability to link an effect to a specific contaminant or contaminant class is highly advantageous from a management perspective, as it provides a clear direction for reducing the beneficial use impairment. Some endpoints are more readily linked to contaminants as the cause than others. Specific biochemical responses, such as acetylcholinesterase inhibition or cytochrome P450 induction, can be quite narrowly linked to particular contaminants or contaminant classes. A clear conceptual linkage of important sources of contamination, exposure, and effect is a major advantage. Other parameters, such as net reproductive success of a population or community composition, can be affected by many factors in addition to contaminants. On the other hand, these more generic indicators are valuable as integrative measures of ecosystem condition, as described below.

General indicator of ecosystem health – While the RMP is focused on contaminants, some of the indicators that are valuable for this purpose might also be of value for more general assessment of the ecological condition of the Bay. Examples include benthic community composition and overall health and reproduction of harbor seals, birds, or fish. The RMP can contribute to a broader assessment of ecosystem condition by supporting sampling of these indicators. These indicators are prime candidates for collaboration with other programs. A good example is the harbor seal work performed to date, in which seed money from the RMP has allowed additional sampling of Bay seals, with a variety of parameters measured by a consortium of investigators.

High signal to noise ratio – Measurement of this indicator should be expected to allow for differentiation between measurement of a response that could be potentially harmful and reference conditions. There should be some data indicating reference conditions for this indicator. This criterion should take in to account field, laboratory, and instrument variability.

Indicators Considered

A wide range of indicators has been considered for inclusion in the EEPS. An initial list of potential indicators was developed through a literature review (Lowe *et al.,* 2001) and a survey of local scientists was performed to develop considerations (Table 1). Discussions of the EEWG focused consideration on the indicators listed in Table 2. It is evident from Table 2 that many potential indicators were excluded from the Pilot Study; brief explanations for excluding these indicators are provided in the Table. For indicators that have been included in the Pilot Study, detailed discussion relative to the selection criteria is provided in Section IV.

For avian monitoring, many species were considered as possible indicators. A table summarizing the pertinent attributes of the different species was prepared by Steve Schwarzbach and used in selecting the most appropriate species for study in the EEPS (Table 3).

2. Criteria and a Strategy for Selecting Indicators for Inclusion in Long Term RMP Monitoring

Selection Criteria

Criteria for selecting indicators for long-term inclusion in the Status and Trends Monitoring Program include the same criteria used for selecting indicators for the Pilot Study, in addition to those described below.

A successful trial in the Pilot Study – Key elements of success should include logistical feasibility, a good ratio of benefit versus the actual cost of the work, continued indication of the likelihood of a significant ecological effect, successful or potentially successful linkage of field studies and controlled studies, a high signal to noise ratio, and an improved conceptual model linking a target contaminant to ecologically significant effects.

A clear reference condition – Reference conditions will be utilized to evaluate both exposure and effects. For exposure indicators, observations will be compared to trends over time and across space. In cases where the reference condition relies on a spatial or temporal comparison, the observed effect should prove to be clearly distinct from a reference condition. A statistically significant difference would be preferred. Where applicable, tissue concentrations will be compared with effects thresholds from the literature to determine the likelihood of effects. For effects indicators (except seals), egg concentrations will be compared to reproductive effects thresholds from the literature. In addition, hatchability studies will complement egg contaminant analysis to examine the linkage between contaminants and reproductive effects. Ideally, each indicator should have a clear evaluation goal that will serve as a trigger to implement a management action.

Contributing to a balanced mix of indicators for the Bay – A mix of indicators will eventually be selected for the "toolbox" mentioned above. This toolbox will provide coverage of the primary taxa, habitats, and timescales of interest.

Selection Process

In the final year of the Pilot Study, while reports on the tested indicators are being prepared, the Workgroup will develop recommendations for RMP exposure and effects work in the years following the EEPS. Workgroup discussions will determine how to express the ratings for each criterion, how each criterion is weighted, and how to combine the ratings for each criterion into an aggregate overall score for each indicator. Summary tables will be prepared to document the ratings and the Workgroup recommendations.

Workgroup recommendations will be provided to the RMP Technical Review and Steering Committees, which will make the final decision on which elements can be included in the RMP on a continuing basis. Input from the Regional Board representatives on these committees will be particularly important in ensuring that the selected indicators address Regional Board information needs.

III. CONCEPTUAL MODEL OF CONTAMINANT EXPOSURE AND EFFECTS FOR THE SAN FRANCISCO ESTUARY

INTRODUCTION

This brief conceptual model of potential sources of contamination to the Estuary and pathways for animal exposure and effects is provided as a framework for a common understanding of exposure and effects and a context for discussion and interpretation of the selected indicators. The rationale and justification for each indicator is described in the following section (summarized in Table 4) to provide information on the relevance of the indicator to RMP and Regional Board management questions and how indicator information will be utilized in management decisions. Study organisms are classified by their place in the food web of the Estuary. Determination of trophic level was made by diet analysis. Species were then classified as primary, secondary, tertiary or quaternary consumers. We also assessed the importance and context of each species as an ecological indicator. The spatial scale of indicators ranges from site-specific (toxicity tests, benthic community assessments, clapper rails) to regional and/or global (toxicity tests, benthic community assessments, seals) and also includes shallow-water (Forster's tern) and deep-water (cormorants) indicators.

SOURCES OF CONTAMINATION

In the Estuary, contaminants may derive from three main pathways: 1) contaminants in dissolved form may enter the Estuary in runoff, discharges, and from the ocean, 2) contaminant-laden sediment particles may be directly transported into the Estuary from its tributary watersheds, or exist in buried layers in Bay sediments and 3) contaminants may enter the Estuary through atmospheric deposition directly from the atmosphere or indirectly by atmospheric deposition of contaminants into the watershed which then is deposited through methods 1 or 2. Dissolved contaminants may adsorb onto the surfaces of mineral particles or into particulate organic material of various origins. Once in the Estuary, adsorption and desorption of contaminants onto minerals and organic material may affect the concentrations in water and sediments at varying spatial and temporal scales. The resulting concentrations (and speciation of contaminants) in water and sediments determine the degree of contaminant exposure to Estuary organisms.

EXPOSURE

Organisms are exposed to contaminants continuously. The contaminant concentration, duration of exposure, and biological availability of the contaminant affects the amount of contaminant accumulated by an organism. The dose of a chemical experienced by an organism is the concentration that is absorbed into their bodies. Routes of exposure vary depending on life stage, feeding and respiratory apparatus, and how the organism interacts with dissolved and particulate contaminant phases. Exposure often occurs via multiple routes; through soft tissues such as gill epithelia, dermal surfaces, or the digestive tract (Forbes *et al.,* 1998; Boese *et al.,* 1990; DiPinto and Coull, 1997; Landrum and Robbins, 1990; and Luoma, 1996). In sediments, exposures are more complex (Figure 1). Exposure may vary depending on the concentration of certain metals and organic chemical compounds in pore water, binding to organic carbon or acid-volatile sulfides, and partitioning between solid sorbed phases and pore water (Luoma, 2000; Thompson and Daum, 1999). Once ingested, particle-sorbed contaminants may be rendered bioavailable by digestive fluids in the gut (Forbes et. al, 1998 and Weston and Mayer, 1998). However not all contaminants are assimilated in the same manner. Proportions of ingested contaminants pass through the gut and are re-deposited in sediment. Some contaminants can be metabolized by the organism. Metabolic rates of contaminants vary by the chemical in question and by organism. Metabolism can reduce tissue contaminant concentrations (Fraser *et. al.,* 2002).

The water column and sediments in the Estuary contain multiple contaminants. Consequently, aquatic and benthic organisms are exposed to numerous trace metal and organic contaminants. These contaminant mixtures create complex exposure regimes. Little is known about how organisms metabolize, accumulate or depurate these complex mixtures of contaminants.

BIOACCUMULATION/BIOMAGNIFICATION

Once assimilated, contaminants may accumulate in various animal tissues. Thus, bioaccumulation is related to dose and the biochemical mechanisms of the organism that interact with different contaminants in different ways. The contaminant may be partitioned into various tissues. Lipophilic organic molecules tend to partition in the fatty tissue of fish and birds (Schnoor, 1996). Bioaccumulation itself is not a toxic effect, but the contaminant may stimulate or impede specific biochemical pathways that may lead to effects. Not all contaminants bioaccumulate. Most metals and trace organics are accumulated. However, the metabolism of non-accumulating contaminants may manifest as effects. In addition to organismal effects, accumulated contaminants in animal tissue have the potential for causing health effects in the wildlife and humans who consume contaminated fish and shellfish.

Biomagnification can occur in aquatic environments. Biomagnification is the progressive increase in body burdens in animal tissues as you move up the food web from primary producers to top-level consumers (Schnoor, 1996). Most of the organisms under study will be secondary and tertiary consumers due to their potential to accumulate contaminants well beyond ambient concentrations and lower trophic level organisms.

BIOLOGICAL EFFECTS

Contaminant effects manifest when the exposure (frequency and duration) and dose (concentration) exceed the assimilative capacity (effects threshold) of the organism. The effect also depends on the mode of toxic action of the contaminant. These modes include genotoxicity (Hose, 1985 and Shugart, 1996), neurotoxicity, narcotic effects (Forbes *et al.,* 1994), immunosuppression (Spies *et al.,* 1990), biochemical inhibition of critical growth or reproductive processes (e.g., macrophage disruption) (Weeks *et al.,* 1990), or stimulation of cellular protection mechanisms, such as lysosomes (Lowe *et al.,* 1995) and stress proteins (Sanders, 1990 and Werner, 1997). As a result of biochemical and sub-cellular processes, tissue (histological) damage may occur (Hinton *et al.,* 1992 and Cross and Hose, 1988). Developmental abnormalities are often observed in organisms exposed to contaminants. Teratogenic effects have been shown in the offspring of laboratory organisms fed diets high in certain contaminants such as selenium (Heinz and Fitzgerald, 1993).

If the dose is high enough, mortality may occur within days (acute effects). However, environmental concentrations are often lower and any biochemical or tissue effects manifest as decreases in critical life functions such as growth or reproduction (chronic effects). These effects may only be subtly observed in individual organisms as decreased growth rates, reduced life span, reduced gamete production, increased rates of fail-to-hatch eggs in birds or teratogenic effects in offspring. Growth, reproduction, and mortality rates are all important population response measures (Connel and DeWitt 1997). Therefore, chronic contaminant exposure on individual organisms may manifest as changes in the local populations. Changes in populations may affect shifts in community composition and change ecological dynamics such as predation and competition. These population shifts shape communities and ecosystems. Often effects are observed as indirect or secondary contaminant effects that occur at the ecological level. For example, an acute contaminant response of an organism may cause it to become locally extinct which then allows a different organism that is more contaminant tolerant to become established. The establishment of the tolerant organism is an indirect or secondary effect of contamination.

The current paradigm for ecological assessments of sediments includes consideration of studies of the effects of contaminants on multiple levels of organization, including cellular, histological, organismal, population, and community-level impacts. This is done because there is little information about all of the linkages among these levels to allow focus on one, or a few of them. Careful indicator selection of assessment endpoints from several representative and important compartments creates a weight-of-evidence for use in management decisions (Cooper and Stout 1985; Stebbing *et al.,* 1992; Forbes and Forbes 1994). The assessments are generally enhanced when combined with contaminant measurements and toxicity information, as well as with forensic and mechanistic studies that identify specific chemicals responsible for organism responses (Schubauer-Berigan and Ankley, 1991).

Additional important areas where information is sorely lacking are 1) the combined effects of mixtures of contaminants and 2) the effects of contaminant stressors in combination with others stressors faced by organisms in the environment. Effects studies traditionally focus on single contaminants, and almost certainly underestimate the actual potential for effects. One way to approach this problem is through monitoring the general health of selected indicator species (Spies 2004). Major advantages of this approach are that it would provide an integrated response to all of the contaminant and non-contaminant stressors faced by a wild population and it could allow detection of effects of contaminants that are not currently routinely analyzed. A disadvantage is that relating the health effects to specific contaminants or other stressors would likely be challenging. Coupling field observations of general health with laboratory studies of health responses to contaminant exposure would be a powerful way of relating contaminants to observed responses. One recent effort to obtain information on responses to realistic exposures is looking at effects of mixtures of contaminants by collecting contaminant mixtures from the environment using semi-permeable membrane devices (SPMDs), then exposing fish early life stages to extracts from the SPMDs (Springman 2004). This work also includes use of a model (the Vitality Model) that allows estimation of the combined effects of contaminant and non-contaminant (in this case, immune challenge) stressors.

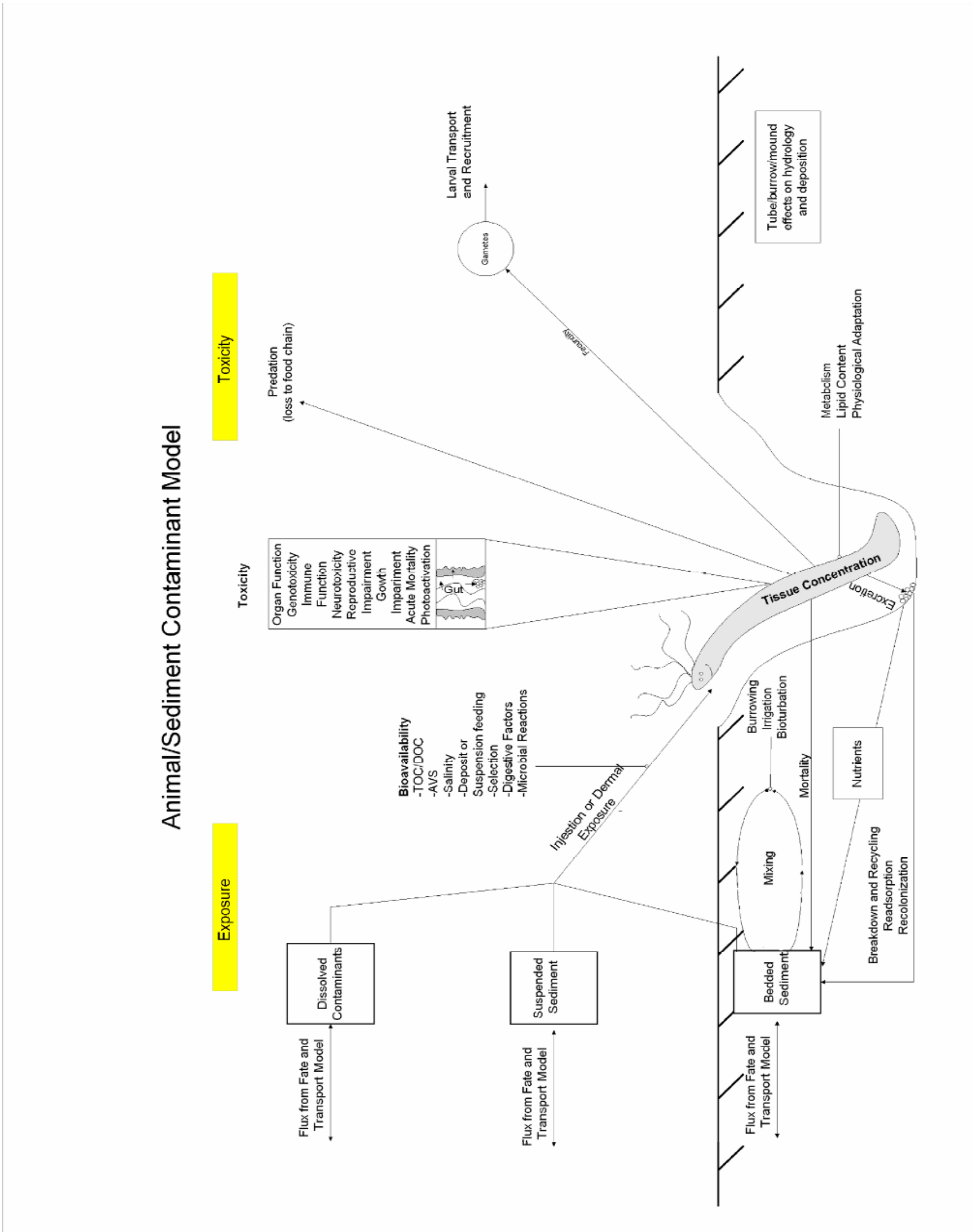

Figure 1. Animal sediment contaminant model. Exposure, assimilation and toxicity of chemical contaminants.

IV. RATIONALE AND JUSTIFICATION BY INDICATOR

BIOACCUMULATION

1. Terns

The terns are being analyzed for egg contaminant concentrations and egg hatchability. The three species being looked at are the Caspian tern, California least tern and Forster's tern. These species feed primarily on fish but are also known to include invertebrates in their diet. Due to a diet high in fish they are tertiary consumers and at risk for increased exposure and accumulation of contaminants. Egg contaminant concentrations can be compared to effects threshold levels determined from laboratory feeding experiments of the same or similar species. Contaminant concentrations in eggs are also a good proxy of contaminant concentrations in females at the time of egg hatching, especially organochlorines and Hg (Ohlendorf, 1993 and Ohlendorf *et al.,* 1988).

Caspian and Forster's tern eggs have been collected randomly from colonies located around the Estuary. Fail to hatch eggs were also collected from nests and analyzed. Only fail-to-hatch eggs were collected for the least terns due to their endangered status. There is only one colony of least terns in the Estuary located at the Alameda National Wildlife Refuge (Adelsbach, per. comm.). All eggs will be analyzed for organochlorines and Hg. In addition to contaminant analysis, nesting sites were also monitored for reproductive success. Nests were monitored for number of eggs/nest, numbers of live and dead chicks/nest and the overall number of nests in a sampling location. A reproductive index is being developed for each study colony. Sample collection was slated for and completed in 2002 and 2003.

1.1. How does the indicator measure relate to various management issues:

1.1.1 RMP Management Questions

Monitoring contaminant concentrations in tern tissue is directly related to the RMP management questions a, b and c (see Section II A above). Monitoring contaminant concentrations in resident eggs will provide a measure of long-term changes in contamination of the estuarine food web. There is also a spatial component that will look at egg contamination in various reaches of the Estuary. The legacy contaminants Hg and PCBs will be looked at in order to determine if these contaminants are at levels that could cause reproductive effects.

1.1.2 Beneficial Uses as outlined by the Regional Board

The Regional Board, through the 1995 Basin Plan, has developed water quality objectives and narrative beneficial use goals for the Estuary. Measuring accumulation of contaminants in the tissues of aquatic species is a key aspect in assessing compliance with the water quality objectives. The Basin Plan also calls for the protection of waters that support the habitat of rare, endangered or threatened species as well as non-listed wildlife. The California least tern is on both the state and federal endangered species list.

1.1.3 Regional Board policy actions or other assessments

The Hg TMDL for the Estuary has been completed and a basin amendment planned for 2003/2004. The PCB TMDL draft was completed at the end of 2003 with a finished report and a basin plan amendment planned for April 2004. The Regional Board will require data on Hg and PCB concentrations in avian eggs as an indicator of the effectiveness of the TMDL.

1.2. Exposure

1.2.1. General Exposure

Resident bird species are exposed to potentially harmful levels of contaminants through their diet. The terns we are looking at are tertiary level consumers. Their diet is mainly piscivorous and therefore they can accumulate high concentrations of certain chemicals. It has been estimated that Hg concentrations in fish are 10^6 to 10^7 times higher than concentrations in water (Schwarzbach and Adelsbach, 2003). These contaminants pass from prey to predator and concentrations may magnify again. Contaminants also pass from a female adult to egg (Ohlendorf *et al.,* 1990). Measuring contaminant levels (Hg) in eggs may serve two purposes: measure regional levels of contamination in the Estuary and provide a basis for linking contaminants to reproductive effects (Schwarzbach and Adelsbach, 2003).

1.2.2. Reference condition

Tern egg concentrations will be compared to reproductive effects thresholds found in the literature as described in section 1.3.2.3 below.

1.3. Effects

1.3.1. Likelihood of finding an effect

Wildlife reproduction can be effected by contaminants. Reproductive effects can include reduced clutch size, embryonic and juvenile deformities, fail-to-hatch eggs, and eggshell thinning. Based on the literature review below, contaminants, Hg and PCBs in particular, have been found to affect the reproductive success of wild birds. Due to the high concentrations of the legacy contaminants Hg and PCBs in the Estuary and their cumulative properties, there is a strong likelihood that tern reproduction could be effected. Measuring egg concentrations may be a better assessment of the links between contaminants and effects than looking at contaminant concentrations in consumer's diet (Fairbrother *et al.,* 1999).

1.3.2. Significance

1.3.2.1. Linkage to population effects

Reduced clutch size, increases in fail-to-hatch eggs and teratogenic and lethal effects in embryos can lead to declines in wild bird populations.

1.3.2.2. Linkage to contamination

There is evidence in the literature that certain contaminants can affect avian reproduction. We can attempt to make a link between contaminants and effects on avian reproduction by looking at feeding studies that document the contaminant levels at which effects occur.

1.3.2.3. Reference Condition

There is a hatchability component to this study. Localized hatchability studies of these three species can be measured against reproductive effects thresholds in the literature. Field studies of Forster's tern eggs have shown that reduced hatching success of this species has occurred when PCB concentrations (totals) are in the range of 8-25 ppm (Hoffman *et al.,* 1996). Other field studies of Forster's tern eggs show embryo mortality, impaired reproductive success and embryonic beak deformities when PCB concentrations (totals) are in the range of 6-26 ppm (Hoffman *et al.,* 1996). Field studies of Caspian tern eggs have shown embryo deformities and mortality and impaired reproductive success at PCB concentrations (totals) in the range of 4-18 ppm (Hoffman *et al.,* 1996). Mercury concentrations can be measured against laboratory studies that have shown reproductive effects in mallards at 0.8 ppm fww (Heinz, 1979), and Fimreite (1971), which found low hatchability in pheasant eggs with concentrations of 0.5 to 1.5 ppm fww. Hatching success of least tern eggs was low in least terns in Southern California in the mid 1990's but most of the loss in eggs was due to predation. Contaminant levels were not high enough to impair reproduction (Hothem and Powell, 2000).

1.4. Literature Review

Piscivorous birds are at a high risk for exposure and accumulation of contaminants due to their trophic position. Legacy contaminants such as Hg and PCBs pose health risks to Estuarine organisms that become contaminated through the aquatic food web. Fish consumption is the primary pathway for methylmercury to enter into higher trophic level wildlife (Schwarzbach and Adelsbach, 2003). It is also the pre-dominant pathway for other contaminants. Biomagnification of organochlorines from fish to tern tissue can range from 40-100 times (Ohlendorf *et al.,* 1985). Accumulation of contaminants can affect reproductive success. In a field study of Forster's tern eggs in the mid-West, egg concentrations of 23 ppm of total PCBs and 37 ppt 2,3,7,8-TCDD resulted in a 50% hatching success (Hoffman *et al.,* 1996). Based on a broad literature review of PCB effects in birds, Hoffman *et al.,* (1996) concluded that total PCB concentrations in the range of 8 to 25 ppm in eggs can lead to decreased hatching success for a range of bird species including terns. Yamashita *et al.,* (1993) found that PCB concentrations (as sum of congeners) at 7 ppm were associated with 25% embryo mortality and deformities in 10% of embryos. The rate of deformities increased from 2% to 10% as concentrations increased from 3600 ppb to 7300 ppb. Reproductive failure of Sterna caspia (Caspian terns) in the Elkhorn Slough National Estuarine Research Reserve (Monterey County) in 1995 was associated with high concentrations of chlorinated hydrocarbons in the tern eggs (Parkin, 1998). In the South Bay three species of bird eggs (*Sterna caspia* (Caspian tern), *Sterna fosteri* (Forster's tern), and *Egretta thula* (snowy egret) showed organic contaminant concentrations similar to those of the night herons, which exhibited lower embryonic weight correlated to PCB concentration (Hoffman *et al.,* 1986). Caspian and Forster's tern eggs were collected from San Francisco and San Diego Bays and Elkhorn Slough and measured for organochlorines and Hg (Ohlendorf *et al.,* 1988). Average PCB concentrations from Caspian tern eggs were significantly higher in the San Francisco eggs than the San Diego and Elkhorn Slough eggs. Average PCB concentrations in San Francisco Bay Caspian and Forster's eggs were 4.85 ppm and 5.65 ppm wet weight, respectively. Mean Total PCB concentrations in least tern eggs collected from the Alameda Naval Air Station and the Oakland Airport were 3.69 and 3.61 ppm fresh wet weight, respectively (Hothem and Zadar, 1995).

Mercury contamination can also have effects in wildlife. The log of adult body weight in terns (Caspian and Forster's) has been positively correlated with mean Hg egg concentrations making a link between adult size and the amount of Hg transferred to eggs (Schwarzbach and Adelsbach, 2003). A recent study of Hg concentrations in San Francisco Bay avian eggs has shown significant differences in Hg concentrations by geographical region and species (Schwarzbach and Adelsbach, 2003). For randomly collected Forster's and Caspian tern eggs, Hg concentrations were highest in South Bay samples. Highest mean Hg concentrations for south bay caspian and Forester tern eggs were 1.18 ppm fww (salt pond A7) and 1.62 ppm fww (salt pond A16), respectively. The median Hg concentrations for these two species were also amongst the highest of all eggs analyzed. Fail-to-hatch least tern eggs collected from central bay had a mean Hg concentration of 0.3 ppm fww. Caspian and Forster's tern average Hg egg concentrations collected from Bair Island were 1.25 and 0.90 ppm wet weight, respectively (Ohlendorf *et al.,* 1988). These concentrations were higher than those found in black crowned night heron and snowy egrets from the same location. The concentrations are above the reproductive effects threshold determined for mallard eggs and are in the range of effects thresholds for pheasant eggs. Caspian and Forster's terns, therefore, may be excellent candidates as indicators of regional contaminant trends. Hothem and Zadar (1995) found mean Hg concentrations in California least tern eggs at the Alameda Naval Air Station (NAS) and Oakland Airport to be 1.75 and 2.05 ppm dry weight, respectively. Two controlled feeding studies that have established accepted thresholds for evaluating risks associated with mercury in wild bird eggs are Heinz (1979), which found low to moderate effects on hatchability of mallard eggs with 0.8 ppm fww, and Fimreite (1971), which found low hatchability in pheasant eggs with concentrations of 0.5 to 1.5 ppm fww. Embryo deformities were also found in mallards fed a high Se diet in laboratory experiments (Heinz and Fitzgerald, 1993). Tern egg mercury concentrations from the Estuary are frequently above 1 ppm, raising concern for possible effects on hatchability.

1.5. Integration

1.5.1. What trophic level does this indicator represent?

All three tern species are tertiary level consumers. They prey mainly on fish but diet may also include invertebrates. As predators, terns are good indicators of the potential for contaminants to bioaccumulate and biomagnify in the Estuary and how this accumulation potentially affects reproductive success.

1.5.2. Will this indicator be able to show temporal trends

A longer-term data set will allow for interpretation of temporal trends in contaminant concentrations in the biota. This will be especially helpful in assessing the Hg and PCB TMDLs for the Estuary. We will have 4 years of data following the 2003 collection period, combining the data from Schwarzbach and Adelsbach (2003) and the EEPS work.

1.5.3. Will this indicator be able to show spatial patterns and at what range will this indicator integrate contaminant exposure or effects?

Recent studies of Hg concentrations in avian eggs from the Estuary show that there is significant geographical variation in Hg concentrations for both Caspian and Forster's terns. Median Hg concentrations were significantly higher in the South Bay than the North and Central regions of the Estuary. Terns are regional indicators and also indicators of contamination in the shallow water habitats of the Estuary. Terns are a good complement to cormorants, which are a good regional indicator for deeper water habitats.

1.6. Additional Indicator Evaluation Measures

1.6.1. Cost (annual, include a sense of scale of the effort)

The tern work included in the Pilot Study was an add-on to an existing USFWS study of organochlorine contamination in tern eggs. The RMP contribution ($40,000) funded detailed field evaluations of tern egg hatchability and analysis of mercury in the tern eggs. The budget for the

USFWS work, covering sample collection and analysis of organochlorines was substantially larger.

1.6.2. Signal to noise ratio

Piscivorous bird eggs accumulate easily measurable concentrations of mercury and organochlorines. The most important reference point for tern eggs is the threshold for concern for toxic effects. Recent work by Heinz (2003) was a major advance in establishing these thresholds for Bay species. Further refinement of the thresholds for terns is needed, but mercury concentrations over 1 ppm are considered elevated.

2. Double-crested Cormorants

The Chlorinated Hydrocarbon Workgroup in 1999 (Davis and Yoon 1999) recommended monitoring for concentrations of bioccumulative substances in the eggs of piscivorous birds. Double-crested cormorants were recommended for several reasons: they are resident; they eat Bay fish almost exclusively, have been the subject of organochlorine studies in the Bay and elsewhere, their eggs are easy to collect (minimal sampling costs), the colonies and eggs are reliably present (in contrast to fish) and are known to accumulate Hg and organochlorines. Piscivorous bird eggs are also valuable for monitoring other persistent, bioccumulative contaminants, such as mercury, selenium, dioxins, and brominated flame retardants. High PCB concentrations in eggs and elevated activity of a liver enzyme biomarker in double-crested cormorant embryos sampled in 1994 and 1995 suggest that PCB exposure in the embryos was sufficient to cause low rates of embryo mortality (Davis *et al.,* 1997; Davis, 1997). We are looking at cormorant eggs to determine if they are a suitable species as a long-term trend indicator of contaminants in the Estuary.

2.1. How does the indicator measure relate to various management issues:

2.1.1. RMP Management Questions

Monitoring contaminant concentrations in cormorant eggs will answer RMP management questions a and b (see Section II A above). Cormorants are good indicators of bioaccumulation of contaminants from the Estuary since their diet consists, predominantly, of fish from the Estuary. We have cormorant egg data collected from as early as 1994. This pilot study would enhance the data set and provide long-term assessment of contaminant changes in the Estuary. Eggs will be collected from Suisun Bay, San Pablo Bay and South Bay and therefore provide information on regional contamination.

2.1.2. Beneficial Uses as outlined by the Regional Board

The Regional Board, through the 1995 Basin Plan, has developed water quality objectives and narrative beneficial use goals for the Estuary. Measuring accumulation of contaminants in the tissues of aquatic species is a key aspect in assessing compliance with the water quality objectives. The Basin Plan also calls for the protection of wildlife habitat in the bay.

2.1.3. Regional Board policy actions or other assessments

The Estuary is currently on the 303(d) list of impaired water bodies for Hg, Se, OC pesticides, PCBs and Diazinon. Cormorant eggs will be analyzed for PCBs, Hg, Se, pesticides and dioxins. The Hg TMDL for the Estuary has been completed and a basin amendment planned for 2003/2004. The PCB TMDL draft was completed at the end of 2003 with a finished report and a basin plan amendment planned for April 2004. The Regional Board will require data on Hg and PCB concentrations in avian eggs as an indicator of the effectiveness of the TMDL. Analysis of egg contaminant concentrations will indicate which contaminants are of concern, document trends in the Bay food web, and highlight spatial distribution of contamination.

2.2. Exposure

2.2.1. General Exposure

Piscivorous birds are exposed to potentially high levels of contaminants through their diet. They are tertiary level consumers and are therefore at risk for ingesting high levels of contaminants through contaminated fish. It has been estimated that Hg concentrations in fish are 10^6 to 10^7 times higher than concentrations in water (Schwarzbach and Adelsbach, 2003). Due to the bioaccumulative properties of certain contaminants such as PCBs and methylmercury, cormorants can bioaccumulate and biomagnify these contaminants to levels far exceeding ambient water and sediment concentrations. Contaminants can affect bird health and reproductive success by increasing embryo mortality and reducing clutch size. Cormorants are resident species and therefore good indicators of contaminant concentrations in the food web (Davis, 1997). They are also strong regional indicators of contaminant levels for particular regions of the Estuary.

2.2.2. Reference Conditions

Cormorants are a regional indicator of contaminants and of deep-water habitat. Data will be utilized in trend analysis of changes in contaminants in the Estuary on a regional scale.

2.3. Effects

2.3.1. Likelihood of finding an effect

This pilot study is an exposure study and will not look at organismal effects of contaminants.

2.3.2. Reference conditions

Although this is not an effects study, concentrations measured in the eggs can be compared to reproductive effects thresholds to determine whether concentrations are at levels that could potentially be causing effects.

There is no field-work component that will measure reproductive success. In the literature, field-work studies have shown that cormorant egg PCB concentrations (totals) in the range of 3.6-6.8 ppm have been associated with embryo mortality and embryonic beak deformities (Hoffman *et al.,* 1996).

2.4. Literature Review

Double-crested cormorants were present in the Estuary in large numbers prior to the 1950s (Cogswell, 1977) but largely disappeared until the 1980's (Stenzel *et al.,* 1991). Since the 1980s populations have further increased, and overall reproductive success and colony size in the Estuary have compared favorably to those reported in other areas (Stenzel *et al.,* 1991). Cormorants are year round residents in the Estuary (Stenzel *et al.,* 1991). Analysis of organochlorines in piscivorous bird eggs has produced the most reliable and powerful data set for long term trend monitoring in the Great Lakes (Stow 1995, Haffner *et al.,* 1997, Hughes *et al.,* 1998, Hebert 1998, Pekarik and Weseloh 1998). Reproductive effects by contaminants in cormorants include eggshell thinning, decreased hatchability, edema, bill defects, growth suppression and induction of cytochrome P450 (Davis, 1997).

Preliminary analysis (unpublished) of the 1999-2001 CISNET double-crested cormorant egg data is now available. PCBs were the only contaminant in cormorant eggs that clearly approached a known threshold for toxic effects (Davis et. al., 2003). Sample year 2000 total PCB concentrations in randomly collected cormorant eggs from the Richmond Bridge site ranged from 2.27 – 2.57 ppm fresh wet corrected, 2001 total PCB concentrations ranged from 2.76 – 3.76 ppm fresh wet corrected. Based on a broad literature review of PCB effects in birds, Hoffman *et al.,* (1996) concluded that total PCB concentrations in the range of 8 to 25 ppm in eggs can lead to decreased hatching success for cormorants, terns, doves, and eagles. Specifically, field studies of double-crested cormorant eggs found that PCB concentrations (totals) in the range of 3.6-6.8 ppm were associated with embryo mortality and embryonic beak deformities (Hoffman *et al.,* 1996). PCB concentrations observed in the CISNET study overlapped the lower end of the effects threshold range, with a maximum of 3.76 ppm fresh weight observed in a composite sample from 2001. The results from the CISNET study indicate that PCB concentrations in San Pablo Bay may be high enough to cause low rates of mortality and deformity in cormorant embryos. Concentrations of DDE in cormorant eggs appear to be just below the threshold for impacts on reproductive success. Embryo mortality due to mercury, selenium, and other measured contaminants appears not to be a concern for cormorants. Concentrations of several contaminants, including PCBs, DDE, and selenium, were significantly higher in 2000 and 2001 than in 1999, suggesting that interannual variation can be considerable.

2.5. Integration

2.5.1. What trophic level does this indicator represent?

Double-crested cormorants are tertiary level consumers. They prey mainly on fish from the Estuary. Cormorants are generalists and mainly piscivores (Wires *et al.,* 2001). Due to their status as a higher trophic level consumer, they are a good indicator of contaminant concentrations in the food web of the Estuary.

2.5.2. Will this indicator be able to show temporal trends?
Double-crested cormorant eggs are expected to be an excellent indicator of long-term trends in food web contamination in the Bay. Data on double-crested cormorant eggs were collected starting in 1994. Collection continued from 1999-2001 through the CISNET project. In 2002 the RMP funded collection of cormorant eggs from three colonies and additional collection is planned for 2004. Analysis of cormorant eggs in the CISNET study and subsequent EEPS studies has laid an excellent foundation for continued long-term trend analysis in this species.

2.5.3. Will this indicator be able to show spatial patterns and at what range will this indicator integrate contaminant exposure or effects?
Double-crested cormorants are resident species in the Estuary. This species preys mainly on fish in the pelagic, or deeper water, areas of the Estuary. As deep-water indicators, cormorants are a good complement to terns, which are representative of shallower water habitat. Since eggs are collected from nests in the San Pablo, Suisun and South Bays they are strong regional indicators of contamination in the food webs of different reaches of the Estuary.

2.6. Additional Indicator Evaluation Measures

2.6.1. Cost (annual, include a sense of scale of the effort)
In 2002 the cost of analysis and sampling was $36,000. This cost includes sample collection and analysis of a comprehensive list of contaminants (organochlorine pesticides, PCBs, dioxins, PBDEs, mercury, selenium). Anticipated 2004 and 2006 sampling and analysis costs are both $36,000/year.

2.6.2. Signal to noise ratio
Piscivorous bird eggs accumulate easily measurable concentrations of mercury and organochlorines. Since cormorant eggs are being sampled as a long-term trend indicator, the most important reference points for the cormorant work in the EEPS are the past observations. Of secondary importance for this element, effects thresholds for cormorants are available from the literature.

3. Diving Ducks

Diving ducks are an important avian contaminant indicator for the Bay. Human consumption advisories for surf scoter and scaup have been in effect since 1986 and

1988, respectively, due to high Se tissue concentrations. The advisory was issued by the California Office of Environmental Health Hazard Assessment (OEHHA) in response to the high Se tissue concentrations found in duck tissue by the California Department of Fish and Game (White *et al.,* 1987, White *et al.,* 1988, White *et al.,* 1989 and Chadwick *et al.,* 1991). The last DFG monitoring effort occurred in 1990. In 2002 the RMP analyzed diving ducks from Suisun, San Pablo and the South Bay for Se, Hg and organochlorines. Due to the 12-year data gap additional monitoring is necessary to determine long-term trends in tissue contaminant concentrations. This study will look at contaminant exposure in diving ducks and provide information for managers to evaluate the need for continuing consumption advice as well as the effectiveness of water and sediment quality guidelines. This study will also help determine if further management action is necessary to decrease the loading of selenium.

3.1. How does the indicator measure relate to various management issues:

3.1.1. RMP Management Questions

Monitoring contaminant concentrations in duck tissue is directly related to the RMP management questions a and b (see Section II A above). Analyses of duck tissue will provide data to assess long-term trends/changes in contaminant concentration as well as spatial variation of contaminants. This study will also provide information on bioaccumulation of contaminants in the biota.

3.1.2. Beneficial Uses as outlined by the Regional Board

The Regional Board, through the 1995 Basin Plan, has developed water quality objectives and narrative beneficial use goals for the Estuary. Measuring accumulation of contaminants in the tissues of aquatic species is a key aspect in assessing compliance with the water quality objectives. Se concentrations in diving ducks will be compared to screening values for protection of human health.

3.1.3. Regional Board policy actions or other assessments

Se is currently on the 303(d) list of impaired water bodies and there is a proposed Se TMDL for the Estuary. A Basin Plan amendment concerning Se is planned for 2010. A long-term Se tissue data set is crucial in monitoring how the Estuary is responding to management actions and if NPDES permits of point source dischargers need revising. In 1998, the SFBRWQCB implemented mandatory reductions of Se in effluents from area oil refineries (Luoma and Presser, 2000). Diving duck Se tissue concentrations can be used as an indicator to measure how reduced loadings to the Estuary are effecting Se accumulation in the biota.

3.2. Exposure

3.2.1. General Exposure

This study will measure contaminant levels in duck breast muscle tissue to determine the safety for human consumption of these species. Breast muscle will be analyzed since this is the tissue most popularly consumed. Se concentrations increase in scoter and scaup tissue over the winter and the collection time may not represent the maximum Se concentrations for these species. The hunting season in the Estuary closes at the end of January and sample collection will occur pre-closure. Although we will not be measuring the maximum concentrations in tissue we will be measuring the maximum risk posed to human consumers of these species.

3.2.2. Reference conditions

This study is to monitor contaminant levels in breast tissue for the safe consumption of scoter and scaup by humans. Contaminant concentrations, Se in particular, will be compared to the OEHHA screening value of 2.0 ppm wet weight established for safe consumption of these two species (Fan *et al.,* 1988). Se concentrations will also be examined for trend analysis, both spatial and temporal.

3.3. Effects

3.3.1. Likelihood of finding an effect

This pilot study is an exposure study and will not look at organismal effects of contaminants. The USGS Western Ecological Research Center is tracking surf scoter along their migratory routes on the Pacific Flyway. Birds will be tracked to nesting grounds in Canada and egg and feather samples will be collected from tagged birds and analyzed for contaminants such as Hg, Cd and Se (http://www.werc.usgs.gov/scoter/plan.html). This study will determine if there is cross-seasonal persistence of contaminants that affect reproductive success. In addition to our collection of surf scoter breast muscle tissue for analysis, USGS will collect the livers and kidneys, of the same birds, and analyze them for metals and also do stable istotope analysis (Susan Wainwright, per. comm.). USGS will collect the gastro-intestinal tract of these birds and determine surf scoter prey. Analysis of liver and kidneys for contaminants will provide information on environmental exposure to contaminants in the Estuary (Ohlendorf, 1993). USGS data will augment the EEPS exposure work and provide an effects facet to this study.

3.3.2. Reference conditions

This study is to monitor contaminant levels in breast tissue for the safe, human consumption of scoter and scaup. We will not compare tissue concentrations to effects threshold concentrations.

3.4. Literature Review

The CDFG studied Se in surf scoter and greater scaup from 1986-1990 and found high concentrations in the tissues of these two species, particularly in the latter years of the study. Selenium concentrations similar to those measured in birds from Kesterson Reservoir in the San Joaquin Valley were found in greater scaup and scoter tissue from the South Bay (Ohlendorf *et al.,* 1986b). In 2002, the RMP sampled both scoter and scaup from Suisun, San Pablo and South Bays and analyzed them for Se, Hg and organochlorines. Analysis of the Se data show that concentrations have decreased since the peak concentration years of the late 1980s. There is, however, high inter-annual variation in the data and additional sampling is necessary to see if recent lower concentrations are indicative of long-term declines in Se levels.

Both scoter and scaup are migratory species that spend winters in and around the Estuary (White *et al.,* 1987). Therefore it is difficult to monitor these species for reproductive effects of contaminants accumulated during their winter habitation. The USGS is tracking migrating surf scoter to their nesting grounds in Alaska and Canada. Eggs and feathers of tagged birds will be collected from the nest and measured for contaminants including Se and Hg. Scoter are a good indicator of temporal changes and spatial variation in Se accumulation and indicators of regional contamination. Currently collection and analysis of scoter and scaup in 2002 (completed) and 2004 is budgeted at $36,000. Se concentrations in surf scoter are a key indicator of Se impairment of beneficial uses in the Estuary. Monitoring Se in waterfowl should be a central element of evaluating the success of water quality attainment for Se. Tracking Se concentrations in key indicator species is especially important now because of the potential for increased loading to the Bay from the San Joaquin Valley via completion of the San Luis Drain (Luoma and Presser 2000).

3.5. Integration

3.5.1. What trophic level does this indicator represent?

Surf scoter and scaup are secondary level consumers. They prey mainly on invertebrate species such as clams, mussels and barnacles (Lovvorn, per. Comm., and White *et al.,* 1987, 1988 and 1989). There is some evidence that the invasive species *Potamocorbula amurensis* accumulates higher levels of Se than other invertebrates (Linville *et al.,* 2002). Surf scoter diet in certain regions of the Estuary (Suisun Bay) has been shown to be 100% *P. amurensis* (Lovvorn, per. comm.). As a secondary level consumer, scaup and scoter are good indicators of the potential for contaminants to bioaccumulate and biomagnify in the Estuary.

3.5.2. Will this indicator be able to show temporal trends?

CDFG collected data between 1986 and 1990 on Se concentrations in various tissues of scoter and scaup. With the recent (2002) RMP analysis of breast tissue for Se, Hg and organochlorines in these species, we are starting to build a long-term data set that will indicate any increases or declines in tissue contaminant concentrations.

3.5.3. Will this indicator be able to show spatial patterns and at what range will this indicator integrate contaminant exposure or effects?

This pilot study will look at contaminant concentrations in 3 regions of the Estuary: San Pablo Bay, Suisun Bay and South Bay. Contaminant concentrations can vary spatially due to loading variations. The San Joaquin valley and oil refineries are two of the main sources of Se to the Estuary. Birds may attain higher concentrations of Se in the Northern reaches of the Estuary due to higher loadings of Se.

3.6. Additional Indicator Evaluation Measures

3.6.1. Cost (annual, include a sense of scale of the effort)

In 2002 the cost of chemical analysis was $17,000, which covered analysis of selenium, Hg and organochlorines in individual ducks (10 ducks from each of three regions) and PCBs and mercury in composite samples from each region. CDFG did the sample collection gratis. Anticipated 2004 costs are $19,000 with CDFG again provided free sample collection.

3.6.2. Signal to noise ratio

Duck breast muscle has been found to accumulate readily measurable concentrations of selenium, mercury, and organochlorines. Since ducks are being sampled as an indicator of impairment and a long-term trend indicator, the most important reference points are screening values for human health concerns and past observations. The signal to noise ratio for comparisons to screening values is good. Significant interannual variability has been observed in selenium concentrations, making the signal to noise ratio for long-term trend evaluation weak.

4. California clapper rail

The California clapper rail is a wetland species that is on both the federal and state endangered species list. Due to its endangered status only fail-to-hatch eggs can be collected for analysis. They dwell and nest in salt marshes that contain pickleweed and cordgrass (USFWS Recovery Plan for California Clapper Rail). The clapper rail is a resident species in the Estuary and is a site-specific indicator. The rail diet is high in invertebrates such as clams, mussels and crabs (USFWS Recovery Plan for California Clapper Rail). Recent analysis of Hg in fail to hatch rail eggs collected from a Central Bay salt marsh (Wildcat Marsh) found elevated concentrations of Hg (Schwarzbach and Adelsbach, 2003). Concentrations of Hg in rail eggs were relatively high compared to other aquatic birds from various regions of the Bay/Delta, and appear to be high relative to the effect threshold for this species, which is particularly sensitive to methylmercury exposure. Embryonic deformities, embryonic hemorrhaging and depressed hatchability may affect clapper rail populations. The Bay clapper rail population is producing fewer young per nest than rail populations from other ecosystems (Schwarzbach *et al.,* 2003), and it is plausible that mercury-induced embryo mortality is a significant factor in this suppression of reproduction. Links to contaminants can be attempted through comparison of contaminant concentrations in field and laboratory studies.

4.1. How does the indicator measure relate to various management issues:

4.1.1. RMP Management Questions

Monitoring contaminant concentrations in clapper rails is directly related to the RMP management questions a, b, and c (see Section II A above). Because the clapper rail is non-migratory, measurement of egg contaminant concentrations will provide a measurement of site-specific contamination. Due to the sampling restrictions on this species, long term temporal or spatial trends may not be possible. Eggs will only be sampled opportunistically. This will provide a measure of contaminant levels in an endangered species in the Estuary and a potential link between contaminants and reproductive effects in this species.

4.1.2. Beneficial Uses as outlined by the Regional Board

The Regional Board, through the 1995 Basin Plan, has developed water quality objectives and narrative beneficial use goals for the Estuary. Measuring accumulation of contaminants in the tissues of aquatic species is a key aspect in assessing compliance with the water quality objectives. The Basin Plan also calls for the protection of waters that support the habitat of rare, endangered or threatened species as well as non-listed wildlife. The California clapper rail is on both the state and federal endangered species list

4.1.3. Regional Board policy actions or other assessments

The Estuary is currently on the 303(d) list of impaired water bodies for Hg, Se, OC pesticides, PCBs and Diazinon. The Hg TMDL for the Estuary has been completed and a basin amendment planned for 2003/2004. The PCB TMDL draft was completed at the end of 2003 with a finished report and a basin plan amendment planned for April 2004. The Regional Board will require data on Hg and PCB concentrations in avian eggs as an indicator of the effectiveness of the TMDL. The Board will also utilize data on contaminant levels in rare and endangered species as part of the TMDLs.

4.2. Exposure

4.2.1. General Exposure

Resident bird species are exposed to potentially harmful levels of contaminants through their diet. Clapper rails are secondary level consumers. Their diet consists mainly of sediment dwelling invertebrates. Clapper rails are wetland residents and may be exposed to higher Hg levels due to high methylation rates in tidal wetland sediments (Schwarzbach and Adelsbach, 2003). Selenium and PCBs are also potential contaminants for clapper rails. Se concentrations in clapper rail eggs from Wildcat Marsh were lower than other species at various locations in the Bay/Delta (Schwarzbach and Adelsbach, 2003) and were below levels of concern in fail-to-hatch eggs from the North and South Bay (Schwarzbach *et al.,* 2003).

4.2.2. Reference condition

Laboratory studies of mallard (Heinz, 1979) and pheasant (Fimreite, 1971) eggs have shown reproductive effects (low hatchability) at Hg concentrations of 0.8 ppm fww and 0.5-1.5 ppm fww, respectively. These effect thresholds will be used in tandem with field hatchability studies of this species in order to assess the potential effects of contaminants on reproduction.

4.3. Effects

4.3.1. Likelihood of finding an effect

The likelihood of finding a significant population level contaminant effect on clapper rails is relatively high. Wildlife reproduction can be effected by contaminants. Reproductive effects can include reduced clutch size, embryonic and juvenile deformities, fail-to-hatch eggs and egg shell thinning. Embryo deformities, embryo hemorrhaging, embryo malpositions, and depressed hatchability rate were seen in fail-to-hatch clapper rail eggs collected from various marshes and through nest monitoring throughout the Estuary (Schwarzbach *et al.,* 2003). In a recent study of contamination in eggs from various species at locations throughout the Estuary and Delta, Hg concentrations in clapper rail eggs were elevated. South bay Hg egg concentrations ranged from 0.18 – 2.51 ppm fww and were highter than north bay concentrations (Schwarzbach *et al.,* 2003).

4.3.2. Significance

4.3.2.1. Linkage to population effects

Reduced clutch size, increases in fail-to-hatch eggs and teratogenic and lethal effects in embryos may lead to declines in wild bird populations. The Bay clapper rail population is producing fewer young per nest than rail populations from other ecosystems (Schwarzbach *et al.,* 2003), and it is plausible that mercury-induced embryo mortality may be a significant factor in this suppression of reproduction.

4.3.2.2. Linkage to contamination

There is evidence in the literature that certain contaminants can effect avian reproduction. We can attempt to make a link between contaminants and effects on avian reproduction by looking at feeding studies that document the contaminant levels at which effects occur. A comparison of laboratory studies and field findings is necessary to make any links between contaminants and reproductive effects. Egg injection studies have proven to be a valuable technique for establishing thresholds for concern for clapper rails and other bird species. Work to date supports a plausible link between mercury exposure and population level impacts.

4.3.2.3. Reference condition

Egg contaminant levels in clapper rail eggs can be measured against reproductive effects thresholds in the literature. There are no effects thresholds for this species but comparison can be made to other species. In addition, only fail-to-hatch eggs will be collected for analysis. Laboratory studies of mallard (Heniz, 1979) and pheasant (Fimreite, 1971) eggs have shown reproductive effects (low hatchability) at concentrations of 0.8 ppm fww and 0.5-1.5 ppm fww, respectively. Field-work will include nest monitoring and hatchability.

4.4. Literature Review

4.4.1. A recent sampling of several species of bird eggs from areas throughout the Bay/Delta showed that fail-to-hatch clapper rail eggs had elevated levels of Hg compared with other species (Schwarzbach and Adelsbach, 2003). Central Bay fail-to-hatch eggs had Hg concentration ranging from 0.6 – 1.06 ppm fww. Levels were interpreted to be high due to foraging behavior of clapper rails that is dominant in tidal wetland sediments where rates of Hg methylation are potentially high. Hg levels found in fail-to-hatch eggs exceeded levels that caused embryo mortality in laboratory studies of other species such as mallards (0.5-1.5ppm fww). Se concentrations were not found to be at levels of concern for this sampling of fail-to-hatch eggs. South Bay average Hg levels in fail-to-hatch eggs were higher than eggs collected in the North Bay (Schwarzbach *et al.,* 2003). South Bay average Hg concentrations from eggs collected in this study (1991-1998) were not different from Hg concentrations in clapper rail eggs analyzed in the 1980s but North Bay averages were half of the 1980 levels. The south bay Hg range in fail-to-hatch eggs was 0.18-2.51 ppm fww and the north bay range was 0.11-0.87 ppm fww. Methyl mercury was an average of 95% of the total Hg in a subset of the clapper rail eggs. Some of the fail-to-hatch eggs were above Hg thresholds for toxicity established for mallards (0.8 ppm fww) and (pheasant(0.5-1.5 ppm fww). There are no laboratory feeding studies on the clapper rail, therefore comparison to other species' threshold levels is required. Other contaminants found at potentially toxic concentrations included boron, chromium, lead and barium. Elevated concentrations of these trace elements were only found in one North Bay marsh (Wildcat). This marsh also had the lowest hatchability rate. Schwarzbach *et al.,* (2001) found mean PCB concentrations of 1.30 ug/g fresh wet weight in fail-to-hatch eggs from 4 south bay locations (n=22). The authors concluded that organochlorines (except possibly PCBs), in the concentrations found in this study, were not a threat to South Bay clapper rail populations. The PCB data was not congener-specific and therefore toxicity comparisons to literature findings were not possible. Eggshell thickness was not significantly different from pre-1932 reference clapper eggshells.

Population studies in North and South Bay marshes showed that hatchability was lower than the established normal hatchability rate of 90% for this species. Hatchability was 65% and 75% for north and south Bay, respectively (Schwarzbach *et al.,* 2003). Hatching success and nest success were also low in both north and south bay marshes. The average nest success in north and south bay marshes was 45% while the average egg hatching success was 39%. The average young produced per active nest was 2.33. Predation accounted for about 33% of lost eggs. Elevated Hg concentrations in fail–to-hatch clapper rail eggs may be above the threshold for decreasing egg hatchability. Decreased hatchability is based on laboratory findings of Hg concentrations in pheasant and mallard eggs and is not a reference threshold for the clapper rail.

4.5. Integration

4.5.1. What trophic level does this indicator represent?
Clapper rails are secondary level consumers. They prey mainly on sediment dwelling invertebrates such as mussels and clams. Clapper rails are non-migratory and are therefore indicators of site-specific contamination in the Estuary.

4.5.2. Will this indicator be able to show temporal trends?
Due to its endangered species status, collection of clapper rail eggs can only occur in fail-to-hatch eggs. Because of this restriction, collection from the same site over time may be difficult. Also the number of eggs/sampling episode will vary and potentially make statistical interpretation difficult. There is a potential for showing temporal trends but it is not an endpoint for this species.

4.5.3. Will this indicator be able to show spatial patterns and at what range will this indicator integrate contaminant exposure or effects?
Clapper rails are an indicator of site-specific contamination. When samples can be collected from more than 1 site in the Estuary, spatial patterns in contaminant concentrations can be determined. Again this is not an endpoint for this species but will be determined when data is available.

4.6. Additional Indicator Evaluation Measures

4.6.1. Cost (annual, include a sense of scale of the effort)
$34,000 has been projected in 2005 for collection and analysis of fail-to-hatch clapper rail eggs.

4.6.2. Signal to noise ratio
Clapper rail eggs appear to exceed the threshold for embryo mortality in this species. However, the threshold is not really well-defined and not that many eggs have been sampled. Further work on both of these fronts would improve the signal to noise ratio. Enabling this evaluation is a high

priority for managing mercury in the Estuary.

5. Pacific Harbor Seals

[The following are excerpts and paraphrasing from 2002/03 Seal reports as part of the EEPS –2002 effort: (Grigg, 2003, Neale, 2003/4, and Nickels, 2003). Full reports available upon request from SFEI]

In many ways, Pacific harbor seals are an ideal indicator species for persistent bioaccumulative contaminants in the Bay. Seals are a good indicator of potential effects on humans because, as mammals, they are more closely related to humans than other species. Seals are apex predators that eat a diet consisting primarily of fish, which renders them highly exposed to contaminants that biomagnify. Seals are opportunistic feeders. Harvey and Torok (1994) found that 11 out of the 14 fish found in seal fecal matter were the most abundant species in the Estuary. The Pacific harbor seals in the Estuary are believed to be mostly resident in their foraging behavior, but some harbor seals do forage in local coastal areas outside the Golden Gate (Nickel and Grigg, 2003). As some seals do forage outside of the Estuary (as far south as Pillar Point and north of Point Reyes (Nickel and Grigg, 2003), contamination levels in seals may not be completely representative of Estuarine contamination. However, they do reflect regional conditions. Bioaccumulative contaminant levels in local populations have been compared to those of other high trophic level predator populations around the world (Grigg, 2003).
The population of harbor seals within the Estuary has not increased in conjunction with increases seen in coastal populations (Kopec and Harvey, 1995). Population levels in the Estuary may not be increasing due to disturbance at pupping sites, limited habitat (no change in the number of haul-out sites) or contaminant effects on seal reproduction. Additionally the population census methods may not accurately capture population conditions as the current method for gathering census data is an annual fly-over of current haul-out sites (Nickel and Grigg, 2003). The EEPS is investigating both an exposure (bioaccumulation) and effects (population) component.

5.1. How does the indicator measure relate to various management issues:

5.1.1. RMP Management Questions

Monitoring contaminant concentrations in seals is directly related to the RMP management questions a, b, and c (see Section II A above). As top predators, seals are at high risk for exposure to bioaccumulative contaminants that may cause measurable effects. Spatial interpretation of contamination levels will be made on a regional/global scale. New bioaccumulation data recently reported (Neale, 2003/4) highlights the temporal changes in PCBs, and DDTs compared to earlier studies from the 1980’s (Riseborough, 1978; Riseborough *et al.,* 1980). This new study also showed that differences in sampling and analytical methods confounds results and makes it difficult to definitively measure changes in tissue levels over time. Consistent monitoring of seal tissues over the long-term would provide a measure of body-burden levels of legacy contaminants in a quarternary level predator and would give managers a way to gauge if management actions taken to mitigate contamination in the Estuary are having an impact on Estuary biota.

5.1.2. Beneficial Uses as outlined by the Regional Board

The Regional Board, through the 1995 Basin Plan, has developed water quality objectives and narrative beneficial use goals for the Estuary. Measuring accumulation of contaminants in the tissues of aquatic species is a key aspect in assessing compliance with the water quality objectives. The Basin Plan also calls for the protection of waters that support the habitat of rare, endangered or threatened species as well as non-listed wildlife. Harbor seals are a protected species under the federal Marine Mammal Act.

5.1.3. Regional Board policy actions or other assessments

The Estuary is currently on the 303(d) list of impaired water bodies for Hg, Se, OC pesticides, PCBs and Diazinon. The Hg TMDL for the Estuary has been completed and a basin amendment planned for 2003. The PCB TMDL is due at the end of 2003. The Regional Board will require data on Hg and PCB concentrations in various food web species as an indicator of the effectiveness of the TMDL.

5.2. Exposure

5.2.1. General Exposure

Field studies have identified the following contaminants or classes of contaminants as cause for concern in seals: PCBs, DDT and derivatives, PAHs, dioxins, furans, chlorobenzenes, chlordanes, Hg, Pb and Cd (Garcia Hartmann, 1997). PBDEs have also recently been found in seal blubber from local San Francisco Bay seal populations (She et. al, 2002). PCBs, as Sum of PCBs in two different studies, were found in the blood of local harbor seal populations at levels exceeding levels found in a laboratory study of seals fed PCB contaminated fish (Kopec and Harvey 1995; Young et. al 1998). Some contaminant levels are still high in seal tissues and have not decreased in the past ten years (Jennifer Neale, per. comm.).

5.2.2. Reference condition

Trend analysis of tissue/blood contaminant concentrations in seals over time and space can provide assessment of overall contamination in the Estuary. Contaminant concentrations in seals can be compared to effects thresholds found in the literature from feeding studies to determine if concentrations in local seal populations are at levels that can lead to effects. Additionally body burden levels can be compared to other seal populations for a relative comparison.

5.3. Effects

5.3.1. Likelihood of finding an effect

There is research on the linkage between contaminants and effects in seals. As in other species it is difficult to conclusively link contaminants in seals to organismal or reproductive effects. In laboratory experiments where captive seals were fed contaminated fish, effects included reproductive failure, immunosuppression, reduced cortisol level, reduced vitamin A level and reduced thyroid hormone levels (Reijnders 1986; de Swart *et al.,* 1994; Reijnders and de Ruiter-Dijkman 1995; Ross *et al.,* 1995; Ross *et al.,* 1996; Van Loverern *et al.,* 2000). Kopec and Harvey (1995) found equal or lower erythrocyte levels in local seal populations when compared to Puget Sound populations and captive populations. Low erythrocyte levels may be linked to environmental stressors, including high contaminant levels. Other indicators of seal health include serum retinol (vitamin A) levels. Again Kopec and Harvey (1995) found that the serum retinol levels of the local seal populations were comparable to the depressed levels found in laboratory experiments with seals fed contaminated fish. However, serum thyroxin (T4) levels of local seals were at normal levels. The PCB levels in Kopec and Harvey's (1995) study were in the range of PCB levels that caused immunosuppression and decreased fecundity in seals fed contaminated fish. Kannan *et al.,* (2000) has estimated that threshold concentrations of seal liver PCB levels where effects may occur range from 6.6-11.0 ug/g lipid weight. In a study of stranded harbor seals, PCB liver concentrations ranged from 7 – 350 ug/g with 3 out of the 4 seals ranging from 140 - 350 ug/g (Kajiwara *et al.,* 2001).

5.3.2. Significance

5.3.2.1. Linkage to population effects

Reproductive effects due to high body burdens of contaminants can reduce population levels. As noted above controlled feeding studies have led to reproductive failure. However, population changes have not been seen using current sampling methods in San Francisco Bay. Certain factors in the Bay may be contributing to this lack of population increase. Changes in climate and oceanographic conditions, habitat and resource limitations (there are a limited number of haul-out sites in the Bay), reduced birthrate and survival, emigration from the Bay and proximity constraints of a large urban environment may all have effects on seal populations (Nickel and Grigg, 2003).

5.3.2.2. Linkage to contamination

As with most species, linking contaminant levels to organism or reproductive effects is difficult. The development of contaminant threshold effects for harbor seals is important in making links between contamination and effects.

5.3.2.3. Reference Condition

Contaminant levels found in wild populations and biochemical and immunosuppression measures can be compared to threshold concentrations from feeding studies and risks for effects can be assessed. Historical population levels are documented for reference and currently show no change in population size. However, it is not possible at this time to link this to possible contaminant exposure effects.

5.4. Literature Review (see also Grigg, 2003 for complete literature review.)

The following is a synopsis of Grigg 2003. Harbor seal populations within San Francisco Bay have not been seen to be increasing in parallel with local coastal harbor seal populations (Kopec and Harvey, 1995). Harbor seals are apex level predators with diets mainly consisting of Estuarine fish (Harvey and Torec, 1994). Fifty-four percent of prey items found in fecal samples of local seals were yellowfin goby. Harbor seals are endpoints for contamination in the Estuary and may be good indicators of Estuarine contamination. Tagging surveys of harbor seals found that most forage in areas between 1 – 20 km of a haul out site (Torok 1994, Nickel and Grigg, 2003) but some seals left the Estuary to areas as far North as Point Reyes and as far South as Pillar Point (Torok 1994 and Kopec and Harvey 1995). This external foraging may compromise some of the interpretation of seals as indicators of contamination in the Estuary. Contaminant concentrations can vary between local pinniped species because of different foraging behavior, diet and migratory behavior (Kajiwara *et al.,* 2001). Contamination concentrations in harbor seals have been documented in the literature. Researchers found that sum of PCBs in blood from local seal populations exceeded sum of PCBs found in seal blood from seals fed contaminated fish from the Baltic/Wadden sea (Kopec and Harvey 1995 and Young *et al.,* 1998). Other contaminants found included DDE, chlordanes and some trace elements. Young *et al.,* (1998) found that 80% of the PCB congeners found in harbor seal blood were the congeners 99, 153, 138, 187, 128, 180 and 170 and that PCB 153 could be utilized as an approximate indicator of the sum of PCB concentrations in seals. The PCB levels found in the Young *et al.,* (1998) study were at levels that caused immune suppression in the captive seals fed Baltic/Wadden sea contaminated fish. PCBs in harbor seal livers were four times higher than DDT concentrations (Kajiwara *et al.,* 2001). PBDEs were also found in local seal populations at the highest levels reported for the species and at increased concentrations over the past ten years (She *et al.,* 2002). PBDE 47 was the dominant congener. Preliminary results of contaminant levels in seal blood indicate that levels of pollutants such as PCBs have not decreased over the past ten years

(Jennifer Neale, per. comm.). Health parameters have also been measured in association with contaminant concentrations in seals. Erythrocyte values were low in local seals when compared to levels in Puget Sound and captive seals (Kopec and Harvey, 1995). This could be linked to 1) disease or environmental contamination or 2) shallow depths of bay dives do not stimulate increased oxygen capacity. White blood cells increase with increasing concentrations of sum of PCBs, DDE and sum of PBDEs (Jennifer Neale, per. comm.). Hemoglobin concentrations decreased with increasing sum of PCBs and DDE.

5.5. Integration

5.5.1. What trophic level does this indicator represent?

Pacific harbor seals are quaternary level predators in the bay food web. They are opportunistic and have a diet dominated by fish. They are an indicator of the endpoint of contaminants in the aquatic food web of the Estuary.

5.5.2. Will this indicator be able to show temporal trends?

Analysis of contamination in local harbor seals dates back to Dr. Risebrough's PCB body burden analyses in 1980 (Grigg, 2003). Though studies have been patchy, there are contamination data in the literature. Seals are an endpoint for bioaccumulative contaminants in the Estuary and it is important to monitor changes in concentrations over time in this species.

5.5.3. Will this indicator be able to show spatial patterns and at what range will this indicator integrate contaminant exposure or effects?

Harbor seal studies from the Estuary will allow a regional/global scale comparison of ubiquitous bioaccumulative contaminants in high-trophic level marine mammals. We would expect to find that seals foraging in the more contaminated estuarine waters would have higher contaminant levels than seals feeding in cleaner coastal waters (Aguilar, 1987). Aguilar *et al.* (1993) looked at patterns in organochlorines residue in long-finned pilot whales and was able to distinguish females from different pods on the basis of this chemical fingerprint. This fingerprinting can assist in delineating the source of contaminant (in-bay vs. coastal).

5.6. Additional Indicator Evaluation Measures

5.6.1. Cost (annual, include a sense of scale of the effort)

Approximately $19,000 was utilized in 2002 for a literature review, a seal tagging and tracking study and to augment an in-progress analysis of organochlorines in seal blood. $10,000 was budgeted in 2003 for potential additional studies and/or grant collaboration with other organizations. Those funds were not spent in 2003 but they will be available in 2004 as potential matching funds for a possible grant.

5.6.2. Signal to noise ratio

Confounding factors in measuring body burden of bioaccumulative compounds include the fact that the contaminants differentially accumulate in different tissue types. Consistent sampling methods and analytical procedures will reduce these confounding factors. On a population scale, it remains to be determined what the best way to measure Estuary populations are as there has been no change in habitat size for harbor seals and the sampling method relies on an annual visual census.

6. Fish Effects

6.1. How does the indicator measure relate to various management issues:

6.1.1. RMP Management Questions

Fish effects studies would address RMP management question c (see Section II A above): which contaminants cause effects in the Estuary?

6.1.2. Beneficial Uses as outlined by the Regional Board

The Regional Board, through the 1995 Basin Plan, has developed water quality objectives and narrative beneficial use goals for the Estuary. One of the beneficial uses is to maintain wildlife habitat. Assessing how wildlife is affected by contamination is important knowledge in the regulation of the sources of contaminants into the Estuary. It can answer the question of how wildlife health, survival and reproduction is affected by contaminants

6.1.3. Regional Board policy actions or other assessments

The Estuary is currently on the 303(d) list of impaired water bodies for Hg, Se, OC pesticides, PCBs and diazinon.

6.2. Exposure

Resident fish species are exposed to potentially harmful levels of contaminants through their diet and direct uptake from water. The RMP already measures contaminant concentrations in the muscle of seven sport fish species as an indicator of chemical exposure in the Bay food web. The use of a fish indicator species in the EEPS will be driven by the need to investigate effects rather than exposure.

6.3. Effects

6.3.1. Likelihood of finding an effect

The likelihood of finding an ecologically significant effect of contaminants on Bay fish populations is unclear. Some recent work suggests that such effects might be possible. Work on Delta smelt by Bennett (2004) suggests the possibility that contaminants could affect this species at the population level by reducing smelt recruitment. Observations of high percentages of sex reversal in salmon in the Central

Valley may be related to contaminant exposure, and represent another scenario in which contaminants could plausibly have significant effects. Previous work in the Bay also suggested contaminant effects on starry flounder reproduction (Spies *et al.,* 1988, Spies and Rice 1988, Spies *et al.,* 1990). PCB concentrations in this study appeared to be high enough to adversely affect fish. Starry flounder females from Central Bay had lower reproductive success than those from San Pablo Bay, and survival of embryos was negatively correlated with concentrations of PCBs in eggs and a maternal liver enzyme biomarker.

6.3.2. Significance

6.3.2.1. Linkage to population effects

As discussed above, contaminant effects could plausibly affect fish at the population level.

6.3.2.2. Linkage to contamination

Linking observed effects in Bay fish populations to particular contaminants is a challenge. Many of the ecologically significant responses that may be observed are non-specific, such as reductions in growth or reproductive success. The case for linkage to particular contaminants can best be made through a combination of field studies and controlled exposures where responses and concentrations observed in the controlled exposures are consistent with field observations.

6.4. Literature Review

Little work on contaminant effects on fish populations in the Bay has been conducted. One major study has been completed in the past 20 years: the work by Spies *et al.,* mentioned above. A few studies have been conducted more recently. One is the study by Bennett on Delta smelt described above. Another was a study of biomarker responses in white croaker (Myers *et al.,* 2002) that was conducted in collaboration with the RMP. This study yielded inconclusive results, and is described further in the Fish Biomarkers of Exposure section below. A study of biomarker responses in Sacramento splittail by Swee Teh of U.C. Davis is presently underway

A workshop was held in February 2004 by the California Bay-Delta Authority (CBDA) on “Contaminant Stressors in the Bay-Delta Watershed”, where a Science Panel chaired by Bob Spies considered development of a strategy for investigating the possible impacts of contaminants on critical species in the watershed. Coordination of EEPS and CBDA activities relating to contaminant effects on fish and wildlife will benefit both programs. The recommendations of the Science Panel should be considered as guidance for the fish effects element of the EEPS as well.

Speakers at the CBDA workshop summarized the current state of knowledge on effects of contaminants in the ecosystem. In regard to effects of contaminants on fish populations, past work was reviewed and potential approaches for future work were discussed. Echoing comments made by the EEPS Advisory Panel at the April 2003 meeting, indicators that can be linked to effects on ecologically important parameters – growth, survival, and reproduction – were favored. Another approach suggested at the Workshop was to monitor the health of selected indicator species as a general indicator of ecosystem health. A major advantage of this approach is that it allows an integrated assessment of the combined influence of exposures to environmentally realistic mixtures of chemical stressors, in addition to the other stressors that also impact wild organisms (disease, starvation, etc.). This approach also provides for effects-based surveillance monitoring, in which effects of new contaminants might be detected at an early stage. This type of indicator could be considered for the EEPS.

6.5. Integration

6.5.1. What trophic level does this indicator represent

Variable, depending on the species selected for study.

6.5.2. Will this indicator be able to show temporal trends

This would depend on trends in the contaminants driving the response.

6.5.3. Will this indicator be able to show spatial patterns and at what range will this indicator integrate contaminant exposure or effects?

The ability to show spatial patterns will depend on the life history of the indicator species selected. Some species, such as shiner surfperch, have proven to be good indicators of spatial pattern in RMP fish contamination monitoring. Other species are more mobile and less informative regarding spatial patterns.

6.6. Additional Indicator Evaluation Measures

6.6.1. Cost (annual, include a sense of scale of the effort)

Fish effects should be a significant component of the EEPS. No investment has been made in this arena to date. We propose to craft a strategy in which approximately $40,000 is applied toward developing a fish effects element in each of the next two years of the EEPS. A strategy for using these funds should be developed by a sub-committee of the EEWG, with input from local experts.

6.6.2. Signal to noise ratio

The signal of contaminant-induced effects on parameters of ecological relevance will likely be not much stronger than the noise, and a challenge to detect.

7. Fish Biomarkers of Exposure

7.1. How does the indicator measure relate to various management issues:

7.1.1. RMP Management Questions

Fish biomarker studies address RMP management question c (see Section II A above). Biomarkers are exposure, and sometimes effects, indicators and in some cases can be linked to contaminants. This type of study will indicate which contaminants or class of contaminants are eliciting biochemical responses in fish.

7.1.2. Beneficial Uses as outlined by the Regional Board

The Regional Board, through the 1995 Basin Plan, has developed water quality objectives and narrative beneficial use goals for the Estuary. One of the beneficial uses is to maintain wildlife habitat. Assessing how wildlife is affected by contamination is important knowledge in the regulation of the sources of contaminants into the Estuary. It addresses the question of how wildlife health, survival and reproduction is affected by contaminants

7.1.3. Regional Board policy actions or other assessments

The Estuary is currently on the 303(d) list of impaired water bodies for Hg, Se, OC pesticides, PCBs and Diazinon.

7.2. Exposure

7.2.1. General Exposure

Many biomarkers are indicators of contaminant exposure, and cannot be directly linked to contaminant effects. For example, vitellogenin production in male fish is a widely used indicator of exposure to endocrine disrupting chemicals. Rainbow trout, carp, salmon, and whitefish are among some of the species that have been studied (Sumpter and Jobling, 1995; Harries *et al.,* 1996; Christiansen *et al.,* 1998; Hansen *et al.,* 1998; Schwaiger and Negele, 1998). However, the production of this yolk precursor protein in males is not known to relate to reduced reproduction, growth, or survival.

Myers *et al.,* (2002) examined three other types of biomarker in a study of white croaker collected in 2000: cytochrome P450 induction, liver histopathology, and fluorescent aromatic compounds (FACs) in the bile. Cytochrome P450 induction is an indicator of exposure to dioxin-like compounds (dioxins, furans, PCBs and others) and PAHs. A linkage of this exposure to effects requires associated laboratory investigations associating a degree of induction with an effect. The histopathological endpoints examined by Myers *et al.,* (preneoplastic lesions and hydropic vacuolation) are possibly indicators of contaminant exposure, but can also be caused by age or other non-chemical stressors. Bile FACs are metabolites of aromatic compounds in the diet, including PAHs, and are a good indicator of exposure to these contaminants. Other biomarkers are also available that deal with reproduction, growth, oxidative stress, immunopathology, and endocrine disruption, and have better linkage to contaminant effects.

7.2.2. Reference Condition

The reference condition in biomarker studies is typically established through evaluation of wild populations from uncontaminated habitats. In cases where establishing a reference condition relies on a spatial comparison, the observed effect should prove to be clearly distinct from the reference condition. A statistically significant difference would be preferred. For example, in the white croaker studies of Myers *et al.,* (2002), a reference population from Half Moon Bay was sampled. Establishing a reference condition that is distinct from the exposed condition is not always easy. In the white croaker study, P450 induction at the reference site was unexpectedly elevated and variable relative to that observed in fish from the Bay.

7.3. Effects

Fish have been extensively used in biomarker studies. Tissues studied include the gills, liver, embryos, blood, and bile. Biochemical monitoring provides a warning signal that enables early detection of responses in contaminant-exposed organisms before structural changes occur, diseases appear, or population shifts take place (Roy and Hanninen, 1993). There are many types of effects indicators including DNA damage, stress proteins, cytochrome P450, acetylcholinesterase, and vitellogenin that have been measured in field and laboratory studies as a response to contaminants such as PCBs, PAHs and dioxins. Biomarkers relating more directly to effects were discussed in the Fish Effects section.

7.3.1. Likelihood of finding an effect

The likelihood of establishing statistically significant biomarker responses in Bay fish would vary depending on the biomarker. If an appropriate assay was available, there would be a strong likelihood of detecting vitellogenin in male fish. Difficulties were encountered in finding a significant elevation of P450 in white croaker. Hydropic vacuolation was common in white croaker, but the cause was unclear.

7.3.2. Significance

7.3.2.1. Linkage to population effects

The linkage of these exposure biomarkers to population effects is weak.

7.3.2.2. Linkage to contamination

The linkages of biomarkers to contamination vary. P450 induction can be linked to exposure to specific classes of chemicals, including dioxin-like compounds and PAHs. Vitellogenin induction occurs in response to exposure to endocrine disrupting compounds. Hydropic vacuolation can occur in response to chemical or non-chemical factors.

7.4. Literature Review

Environmental biomarker analyses in the San Francisco Bay-Delta have generally been limited to fish and birds (e.g. flounder, sanddabs, croaker, and cormorants). Werner *et al.,* (1996; 1998) studied heat stress proteins in amphipods in sediment bioassays from the Estuary.

Since biochemical monitoring has become an important component of environmental monitoring, it is important to understand how each indicator varies with the reproductive cycle of the organism, seasonal changes, and among species (Roy and Hanninen, 1993). Synergistic and antagonistic compounds can also influence biomarker responses and should be considered (Molven and Goksoyr, 1993; Bocquene *et al.,* 1995; Delong and Rice, 1997). Biomarkers include genotoxic indicators, detoxification enzymes, stress proteins, and immune and blood system changes. Biomarkers provide information about the causes of biological effects by evaluating multiple indicators and determining which indicators are compromised (Adams, 2001). For example, PAHs are generally inducers of the cytochrome P450 enzyme system, while organophosphate exposure from agricultural activity is expressed in acetylcholinesterase inhibition.

The cytochrome P450 family of enzymes aid in the metabolism of potentially harmful, water-insoluble hydrocarbons. There are many types of P450 enzyme systems that are studied because they are good indicators of exposure to PAHs, PCBs, dioxins, and to a lesser extent organochlorine pesticides (Spies *et al.,* 1988; Rattner *et al.,* 1993; 1996; Spies 2001). Organisms from many trophic levels have been studied including fish (Fent and Stegeman, 1993a, 1993b; Billiard *et al.,* 1999, 2000; Hawkins *et al.,* 2000).

A number of field studies have successfully used AChE inhibition in fish as a biomarker for organophosphate and carbamate insecticides in the estuarine environment. Acetylcholinesterase (AChE) is a regulatory enzyme in the neuromuscular junction (Bocquene *et al.,* 1995; Hughes *et al.,* 1997; Fulton and Key, 2001). Cholinesterase (ChE), AChE, and butyrylcholinesterase (BCE) activity may be decreased by mercury and methylmercury exposure (Wolfe *et al.,* 1998).

Based on observations such as the occurrence of hermaphroditism and feminization of male fish, many investigations focus on fish as indicator organisms for xenoestrogenic compounds within the aquatic environment. Both in vitro assays and in vivo approaches have been developed to evaluate estrogenic effects of these toxicants.

7.5. Integration

7.5.1. What trophic level does this indicator represent

Trophic level would depend on the species studied.

7.5.2. Will this indicator be able to show temporal trends

Temporal trends are not an endpoint in this study.

7.5.3. Will this indicator be able to show spatial patterns and at what range will this indicator integrate contaminant exposure or effects?
There is potential for showing spatial patterns of effects when samples are collected from various reaches of the Estuary.

7.6. Additional Indicator Evaluation Measures

7.6.1. Cost (annual, include a sense of scale of the effort)
$63,000 was allocated to do biomarker work in fish sampled in the 2003 sampling effort. This work was not funded due to the ambiguous results obtained in the 2000 white croaker study and budget limitations.

7.6.2. Signal to noise ratio
The signal to noise ratio varies. For P450 induction in white croaker in 2000, the signal was not detectable above the noise. For vitellogenin, there is no noise, and this is one of the attractive features of this biomarker. For hydropic vacuolation, there is considerable noise.

8. Aquatic and Sediment Toxicity

Laboratory toxicity testing of environmental samples is an established monitoring tool for evaluating environmental condition, and the RMP has been monitoring for toxicity in both water and sediment samples since 1993 using EPA protocols on estuarine laboratory test species. While toxicity tests provide information about whether an environmental sample is toxic to laboratory species, it is important to relate this to the environment.

The RMP has been investigating possible causes of persistently toxic species by performing Toxicity Identification Evaluation studies (TIEs) on samples that are significantly toxic. TIEs are tests that, through a series of chemical chelating and/or other steps, removes toxic agents in an ambient sample allowing (by a process of elimination) identification of the contaminant class/es that may be causing the observed toxicity. The RMP has found that samples from the South Bay and Suisun Bay, that have been persistently toxic to bivalve embryos, are no longer toxic when acid-soluble compounds (such as metals) are removed.

Another way to link laboratory tests to environmental conditions is to understand the relative sensitivities between the laboratory test species and resident species to specific contaminants. Dose-response studies provide this comparison.

Dose-response studies are controlled laboratory tests that through a series of tests of increasing contaminant concentrations, determines the concentration at which 50% of the test organisms die (LC-50) for a particular contaminant and test species. While there are substantial references for LC-50s for fresh water test species, very little information exists on LC-50s for estuarine species (Brian Anderson (UCDavis), pers. com.). For example, we do not know if our current laboratory test amphipod species for sediments (*Eohaustorius estuarius*) is as sensitive to contaminants as the resident amphipod (*Ampelisca abdita*). We also do not know if they show similar sensitivities

to the same contaminants.
The Exposure and Effects Pilot Study plans to augment the RMP's past and current toxicity work by 1) comparing non-resident laboratory species with resident species in dose-response tests to gauge their relative sensitivities (this is a *calibration exercise*), 2) developing LC-50s for estuarine species for a few key contaminants of concern (this will help in interpreting the TIEs and what the possible causes for the observed toxicity might be).
By augmenting toxicity testing with dose-response studies for estuarine species and by calibrating the laboratory test species to resident species, we may have more success in extrapolating laboratory toxicity results to ambient conditions.
The dose response study currently funded by the RMP (2004) and a SFEI PRISM grant (awaiting funding) will include testing of copper, PAHs and pyrethroids (a class of pesticides that is increasingly being used as chlorpyrifos is being phased out) on the RMP (and EPA) laboratory test amphipod *Eohaustorius estuaries,* a resident amphipod *Ampelisca,* and (possibly) a second resident species. The Toxicity Work Group was considering investigating a meiobenthic copepod species and/or a resident bivalve. Recent discussions with Tom Chandler (University of South Carolina) showed that LC50s have been developed for the copepod species *amphiascus* for some of the contaminants of concern in the Estuary (Brain Anderson, (UCDavis, pers. com.)).

8.1. How does the indicator measure relate to various management issues:

8.1.1. RMP Management Questions

Toxicity testing is an effects indicator and answers RMP questions b and c (see Section II A above).

8.1.2. Beneficial Uses as outlined by the Regional Board

The Regional Board has developed water quality objectives to meet the 'beneficial use' goals for the Estuary that include narrative toxicity objectives (1995 Basin Plan). One of the beneficial uses is to measure contaminant effects on survival and reproduction.

8.1.3. Regional Board policy actions or other assessments

The Estuary is currently on the 303(d) list of impaired water bodies for Hg, Se, OC pesticides, PCBs and Diazinon. RMP's toxicity testing played a role in the federal EPA limiting the use of the pesticides diazinon and chlorpyrifos in certain applications. TIEs can be utilized to pinpoint contaminants of concern in certain geographic locations. Dose-response studies will provide needed information about the sensitivities of estuarine species to key contaminants of concern.

8.2. Exposure

8.2.1. General Exposure

Exposure is assumed as a precursor to toxicity.

8.2.2. Reference condition

Contaminant concentrations will not be measured in toxicity test organisms.

8.3. Effects

Exposure to contaminants or groups of contaminants can have effects on estuarine organisms. Toxicity tests specifically look at how sediment and water effect the survival and development of test species. Test species include larval fish, bivalve embryos and amphipods. The combination of toxicity tests, dose-response tests and TIEs provide a stronger weight-of-evidence that certain contaminants or classes of contaminants are linked to effects in estuarine organisms. Dose-response tests provide a LC-50 measurement of toxicity. LC-50s are readily available for freshwater species but are lacking for estuarine species. This study would provide more information on toxicity to resident and non-resident estuarine species. Since aquatic toxicity has appeared to decrease in the past few years, more emphasis may be put on sediment toxicity and benthic organisms. Sediment toxicity has been shown to be widespread and pervasive since the early 1990's.

8.3.1. Likelihood of finding an effect

We have seen toxicity effects (mortality and reduced development) in both aquatic and sediment testing, particularly during the wet season. Mortality and reduced development of test species has already been established in a certain percentage of samples. Aquatic toxicity, however, has seemed to decline.

8.3.2. Significance

8.3.2.1. Linkage to population effects

Effects that may be determined include organism mortality and % normal development of embryos. These response measures have a direct effect on population size.

8.3.2.2. Linkage to contamination

The development of contaminant threshold effects for estuarine test species (through dose-response studies) is important in making links between contamination and toxic effects. By combining toxicity tests with TIEs and dose-response studies, we hope to begin to link observed mortality and reduced development in laboratory tests to particular chemicals or class of chemicals. Contaminant levels found in Estuary sediments can then be compared to threshold concentrations (LC-50s) identified for key estuarine species and risks for effects can be assessed.

8.3.2.3.Reference condition

Toxicity endpoints may be compared to control samples using t-test comparisons for significant difference.

8.4. Literature Review

Benthic organisms have generally sessile habits and are therefore good indicators of localized, time integrated contaminant effects. They are the most commonly used bioassessment indicators in the U.S. (Southerland and Stribling, 1995) and are a key component of the ecosystem. They link sediments to the aquatic food web, provide food for bottom feeding fish and birds, and facilitate other important sediment functions, such as nutrient and carbon flux, by their burrowing and feeding activities. Most macrobenthos are not very mobile and therefore must respond to a variety of natural and anthropogenic changes including rapid changes in flow, salinity, turbidity, dissolved oxygen, xenobiotic contamination, and the immigration of new species.

Laboratory toxicity tests often use benthic organisms as the indicator species. It is important to link laboratory tests to local field conditions in order to provide the ecological context. Foe (1995) emphasized the need to use resident taxa along side well established toxic indicators used in the laboratory in order to bridge this gap. Some resident taxa (found in the Estuary) have been used in laboratory bioassays and could possibly be used as resident indicators (Bailey, 1993; Bailey et al., 1994; Swartz et al., 1994; Anderson et al., 1998; 2001a; Fairey et al., 1998; Thompson et al., 1999; Ogle and Gunther, 2000). These include the crustaceans (e.g. Mysidopsis bahia, Ampelisca abdita, Neomysis mercedis, and Hyalella azteca) and bivalves (e.g. Corbicula fluminea).

Eohaustorius estuaruis is a well-established laboratory amphipod commonly used in sediment bioassays in several studies in the Bay including the RMP Status and Trends Program, and the Lauritzen Channel, a Superfund remediation site (Swartz et al, 1994; SFEI, 1998; Hunt et al., 1998; Anderson et al., 2000; 2001a).

Ampelisca abdita have been collected throughout the estuary. Preliminary studies to develop methods for their use in toxicity tests have shown inconsistent responses to contaminants between organisms from different sources (Anderson, personal communication). *Ampelisca abdita*, a resident amphipod in the San Francisco Estuary, is thought to be sensitive to contamination (Swartz et al., 1994; Ferraro and Cole, 1997), and has been used in sediment bioassays. However, more information is needed to verify the sensitivity of *Ampelisca abdita* because they have been found at sites with high sediment contaminant concentrations, and different laboratory sources of *Ampelisca abdita* have performed inconsistently in laboratory bioassays. Other amphipod species, such as Corophium acherusicum, are also considered to be sensitive to pollution (Pearson and Rosenberg, 1978; Tetra Tech, 1990; Swartz et al., 1994; Ferraro and Cole, 1997; Flemer et al., 1997). Unusual, transient population explosions of *Corophium acherusicum* have been observed in the Estuary and the

mechanisms of their occurrence is not well understood (SFEI, 2000). The amphipod *Grandidierella japonica* is considered pollution tolerant (Swartz et al., 1994; Carr et al., 1996; Ferraro and Cole, 1997).
Swartz et al. (1994) found that amphipod abundance (excluding *Grandidierella japonica*) decreased significantly along a DDT and dieldrin contamination gradient in the Lauritzen Channel (Richmond, CA). Sediments from the gradient were positively correlated with sediment toxicity to the commonly used laboratory amphipod *Eohaustorius estuaries*, and to resident estuarine and freshwater amphipods *Rhepoxynius abronius*, and *Hyalella Azteca* respectively, thus strengthening the link between laboratory tests and in situ ecological condition.
Since 1993 the RMP Status and Trends monitoring has assessed sediment toxicity using larval mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) in sediment elutriate survival tests, and more recently using undisturbed sediment cores (sediment-water interface exposures, Phillips et al., 2000; Anderson et al., 2001). Sediment TIE procedures developed by the Environmental Protection Agency were used to demonstrate that metals were most likely responsible for inhibited bivalve embryo-larval development in whole sediment (sediment-water interface) and sediment elutriate samples from the three North Bay RMP river stations (Anderson et al., 2001). Dose response evaluations have been determined for tributyltin along with genotoxic potential for cytogentic damage (Jha et al., 2000). Solé et al. (1995) studied seasonal cytochrome P450 and antioxidant enzyme responses in *Mytilus galloprovincialis* and found that they correlated with concurrent contaminant body burden levels of PAHs, PCBs, DDTS, and Lindane (when data were normalized to lipid weight).

8.5. Integration

8.5.1. What trophic level does this indicator represent

Toxicity organisms include primary through tertiary consumers (larval fish are predators).

8.5.2. Will this indicator be able to show temporal trends

Yes, (see Thompson et al. 1999). This indicator (incidence of toxicity) is a well established tool to show both spatial and temporal patterns.
The episodic toxicity monitoring study has shown a decrease in aquatic toxicity to laboratory test species in several Estuary tributaries since 1996. The RMP sediment toxicity monitoring has shown little change in the incidence of toxicity since it began in 1993. This indicator (incidence of toxicity) is a well established tool to show both spatial and temporal patterns.

8.5.3. Will this indicator be able to show spatial patterns and at what range will this indicator integrate contaminant exposure or effects?

The RMP has shown spatial and temporal patterns in both aquatic and sediment toxicity studies at local scales (site specific and by tributary) over the past ten years (Pulse, 2003).

8.6. Additional Indicator Evaluation Measures

8.6.1. Cost (annual, include a sense of scale of the effort)

The RMP currently spends ~$220,000 annually on the Estuary Status and Trends monitoring (27 sediment samples throughout the Estuary (~74K)) and the Episodic Toxicity monitoring in the tributaries (~6 tributaries monitored about 5 times during winter storm-runoff events (~146K)).

EEPS has budgeted $60,000 in 2004 to perform dose-response tests related to the RMP sediment toxicity test species and resident estuarine species. Additional funding through a PRISM grant will augment this effort by another $60,000. $60,000 has also been budgeted for 2005 to perform dose-response tests in estuarine resident and RMP laboratory aquatic species.

8.6.2. Signal to noise ratio

Toxicity tests have a high signal to noise ratio and use statistical t-tests to determine samples that are significantly toxic.

9. Benthic Macrofauna

Benthic invertebrates are an important ecosystem component. They transform sediment bound contaminants into the food chain, and are an important food source for fish and birds. They also facilitate geochemical transformations of sediment-bound nitrogen and carbon. Benthos are the most commonly used biological component in bioassessments in the US (Southerland and Stribling, 1995) and are used in all major National and Regional monitoring programs. Through decades of study and usage worldwide, the responses of benthos to sediment contamination are well documented (Long *et al.,* 1995). A variety of species, population response, and community metrics are commonly used in benthic assessments (Thompson and Lowe, in press).

Benthic community assessments are commonly used along with other sediment indicators, such as toxicity, contamination, and bioaccumulation in a weight-of-evidence about sediment condition (Long and Chapman, 1985 and Chapman *et al.,* 1987). Benthic species differ in their sensitivity or tolerance to elevated contamination in the environment. Through a multimetric assessment of the benthic community, it is possible to determine if that community is adversely affected by elevated contamination. Because environmental contamination is usually a mixture of chemicals, the benthic community assessments integrate and respond to complex contaminant mixtures. Benthos also respond to variation in non-anthropogenic environmental factors such as salinity, grain-size and TOC. However, most

assessment methods account for those differences based on study and understanding regional benthic responses.
For the above reasons the State of California has recently begun a project to develop biological criteria for estuarine benthos. A benthic assessment method for SF Estuary has been developed, reviewed, and published (Thompson and Lowe, in press). It uses an Index of Biotic Integrity (IBI), multimetric indicator approach, comparing abundances of 4 or 5 benthic metrics in the two main benthic assemblages of the Estuary, to reference ranges. Each benthic assemblage has characteristic salinity and sediment type differences. The assessment method determines the degree of impact from none to severe due to exposure to sediment contamination.

9.1. How does the indicator measure relate to various management issues:

9.1.1. RMP Management Questions

Benthic assemblage is an effects indicator and answers RMP questions b and c (see Section II A above).

9.1.2. Beneficial Uses as outlined by the Regional Board.

Benthic indicators can address narrative Beneficial Uses for biological integrity, reproduction, support for important fish and birds, and toxicity. The condition of, and impacts to sediment habitats can be assessed using benthic indicators.

9.1.3. Regional Board policy actions or other assessments

As part of the State Biocriteria and Sediment Quality Guideline projects, and WEMAP, this assessment method will be further tested and intercalibrated with other west coast assessment methods, and considered for use in other west coast estuaries. Some form of benthic biological criteria will be implemented by the State, and the RMP should continue to support participation in the development of useful benthic assessment methods. The RMP can be proud that the benthic pilot study has taken a leading role in these projects.

9.2. Exposure

9.2.1. General Exposure

Benthic organisms are directly exposed to contaminants in both water and sediments as described in section III. Exposure may occur through several different mechanisms depending on geochemistry and on the organism's morphology, life habits (tubiculous, burrowing, etc.) and feeding mode. However, all macrobenthos live in, or on sediment and most ingest some sediment (Forbes *et al.,* 1998). Understanding animal-sediment relationships is a key step in understanding contaminant mobilization into the food-chain and exposure to higher trophic levels. Once ingested some contaminants will bioaccumulate in animal tissue. These organisms then serve as prey for other animals and thus begins the process of biomagnification of contaminants.

9.2.2. Reference condition

9.3. Effects

Biological effects to the organism may occur depending on the duration and dose of exposure and on the contaminant. As described in III, the effect of contaminant exposure depends on the mode of toxic action of each contaminant. Effects may be manifest in individual organism's cells, tissues, metabolic functions, or reproduction. For monitoring benthic organisms, usually those effects are observed as increases or decreases in specific contaminant tolerant or sensitive species in local populations having an overall effect on community composition.

9.3.1. Likelihood of finding an effect

Initial applications of this method distinguished sites with impacted benthos from reference samples in San Francisco Estuary. Impacted benthos exist at some Estuary margin and tidal marsh sites, but only at a few deeper Estuary locations.

9.3.2. Significance

9.3.2.1. Linkage to population effects

This linkage is implied for most benthos where local population effects may be observed related to sediment contamination. Since most of SF Estuary has elevated sediment contamination, a hypothesis exists that there may be extinctions of some historical taxa that are sensitive to contamination.

9.3.2.2. Linkage to contamination

Impacted benthos in the San Francisco Estuary have been shown to be clearly linked to sediment contamination. However, benthos in the mesohaline assemblages may also be influenced by elevated TOC.

9.3.2.3. Reference Conditions

Reference conditions for the San Francisco Bay have been identified. Since there are no locations free of sediment contamination in the Estuary, and sediment toxicity is widespread and persistent, there may be no truly unimpacted benthic sites in the Bay. Further, no other estuaries along the central California coast are similar enough to San Francisco Estuary to be suitable as reference locations. Thompson and Lowe (in press) used a screening procedure to identify benthic samples that showed no evidence of benthic impacts based on co-occurring sediment toxicity data and on expected benthic reference conditions as reported in the literature from other areas. They identified a set of benthic samples from the San Francisco Estuary that appeared to represent the least impacted (Reynoldson *et al.,* 1997) benthic conditions in the Estuary.

9.4. Literature Review

Adaptations of the well-established IBI used extensively in fresh water (Karr and Chu, 1999), have been applied in marine and estuarine areas along the East Coast of the US (Weisberg, *et al.,* 1997; Van Dolah *et al.,* 1999), in California's estuaries (Anderson *et al.,* 2001; Jacobi *et al.,* 1998), and in San Francisco Bay (Chapman *et al.,* 1987; Hunt *et al.,* 2001; Thompson and Lowe, in press). In these assessments, benthic indicators (e.g., species diversity, abundance of key taxa) were used in a multimetric index to distinguish impacted from reference benthic conditions. Another assessment approach used multivariate analyses of species composition and abundances to describe assemblage patterns and responses to abiotic variables in the Gulf of Mexico and on the East Coast (Engle *et al.,* 1994). The Benthic Response Index (BRI), developed for southern California [Smith *et al.,* 2001; Bergen *et al.,* 2000), and the benthic assessment methods proposed for Puget Sound (SEA, 1996) combined the multimetric and multivariate approaches described above.

9.5. Integration

9.5.1. What trophic level does this indicator represent?

Benthic organisms include primary through tertiary consumers (some benthic organisms are predators).

9.5.2. Will this indicator be able to show temporal trends?

Yes, many benthic taxa have life histories that produce seasonal abundances (Nichols and Thompson, 1985). Estuary benthos have been shown to respond to changes in salinity as well as sediment type caused by seasonal freshwater inflows.

9.5.3. Will this indicator be able to show spatial patterns and at what range will this indicator integrate contaminant exposure or effects?

9.6. Additional Indicator Evaluation Measures

9.6.1. Cost - $15,000 was allocated in 2002 to evaluate data from NOAA, RMP, EMAP and other studies.

9.6.2. Signal to noise ratio

Table 1. General suite of exposure and effects indicators to be evaluated in the RMP's Biological Effects Pilot Study.

EFFECTS INDICATORS	**Trophic Level**	**Description**
Histopathology of fish	high	Liver and gonad evaluations in conjunction with fish bioaccumulation and biomarker studies provide a solid weight of evidence for biological effects of contamination.
Physiology of seals	high	Would evaluate overall health measures of a high tropic lever organism. Would provide a regionally integrated ecological effects measure.
Toxicity (invertebrates)	low	Toxicity component that include resident and important prey species provide site-specific information of possible effects. Gradient studies, insitu studies, and tolerance testing that complement studies that have already been done would provide necessary background information.
Toxicity (Fish larvae)	high	Menidia are currently proposed as part of the RMP Episodic Toxicity component. We would like to consider using a rare & endangered species to address beneficial use questions.
Bird egg hatchability and/or other reproductive measures	high	Birds integrate regional exposure to certain contaminant groups and based on their feeding and behavioral habits provide information about different modes of exposure within the food web.
Benthic Community	low	Benthic evaluations are a commonly used tool for both site-specific and regional monitoring of ecological effects. The NOAA-EMAP San Francisco Bay study of 2000-01 will continue the development of a SF Estuary specific benthic assessment protocol.
EXPOSURE INDICATORS	**TROPHIC LEVEL**	**NOTE**
Biomarkers: P450, vitellogenin, macrophage aggregates, acetylcholinesterase, Comet test, etc.	high	These tests help to evaluate possible cause of effects by providing information about exposure to specific contaminant groups. Could be studied in invertebrates, fish and/or birds.
Bioaccumulation of contaminants in bird eggs, fish tissue, benthic prey species, and seals	low & high	These tests help to evaluate possible cause of effects by providing information about exposure to specific contaminant groups. For seals, we would look at contaminant levels in blood (or fat tissue).
TIEs	low	This is an ancillary measure for toxicity testing that helps provide information about exposure.

Table 2. Indicators considered for inclusion in the Pilot Study. Bold items have been selected for inclusion in work performed to date. Items with an asterisk were performed at no cost or highly reduced cost to the RMP. "S&T" indicates work performed as part of RMP Status and Trends monitoring.

SPECIES	INDICATOR	BRIEF EXPLANATION FOR INDICATORS NOT INCLUDED
Diving Ducks	**Contaminants in muscle**	
	Contaminants in liver	Effects on ducks themselves a lower priority, livers archived for possible future analysis
	Reproduction	Difficult due to migration, lower priority, work being done by USGS
	Body condition *	
Potamocorbula	Contaminant concentrations	Not a high enough priority to fit existing budget
	Reproductive status (gonad histology)	Not a high enough priority to fit existing budget
	Condition index	Not a high enough priority to fit existing budget
Cormorants	**Contaminants in eggs**	
	Egg hatchability	Large effort, low probability of significant effect
	Effects in embryos	Large effort, low probability of significant effect
Forster's Terns	**Contaminants in eggs ***	
	Egg hatchability	
Clapper Rails	Contaminants in eggs	Logistically infeasible in 2002 and 2003
	Egg hatchability	Logistically infeasible in 2002 and 2003
Least Terns	**Contaminants in eggs ***	
	Egg hatchability	
Snowy Plovers	Contaminants in eggs	Relatively weak link to Bay food web, lower likelihood of significant effect
	Egg hatchability	Relatively weak link to Bay food web, lower likelihood of significant effect
Peregrine Falcons	Contaminants in eggs	Logistically challenging
	Egg hatchability	Logistically challenging
Harbor Seals	**Population status ***	
	Juvenile survival	Good area for future work
	Reproductive rate	Low likelihood of contaminant effect

SPECIES	INDICATOR	BRIEF EXPLANATION FOR INDICATORS NOT INCLUDED
	Contaminants in blood *	
	Contaminants in blubber	Matrix too variable for trend analysis
	Immune system effects *	
	Blood chemistry *	
White croaker	**Contaminants in muscle (S&T)**	
	Contaminants in liver	Not a high enough priority to fit existing budget
	Biomarkers (liver histo, gonad histo, P450, bile FACs) *	
	Vitellogenin in male fish	Not linked to population level impact
	Growth	Possible area for future work
	Reproduction	Possible area for future work
	Survival	Possible area for future work
Shiner surfperch	**Contaminants in muscle (S&T)**	
	Contaminants in liver	Not a high enough priority to fit existing budget
	Biomarkers	Not a high enough priority to fit existing budget
	Vitellogenin in male fish	Not linked to population level impact
	Growth	Possible area for future work
	Reproduction	Possible area for future work
	Survival	Possible area for future work
Resident Species Toxicity	Aquatic toxicity	Thought that aquatic toxicity in the Estuary may best be monitored in the freshwater regions of local tributaries during storm events.
	Dose-Response studies	
	Sediment toxicity	Thought that EPA lab species should not be changed to resident species, but that a “calibration” study would provide needed information to relate laboratory results to estuarine populations.
Benthic community evaluations	**Community composition**	

Table 3. Attributes of bird species considered in selecting candidates for monitoring.

Species	Local vs global forager	Management importance	Toxic response	Bioaccum ulation potential	Geographic distribution	Habitats represent ed	Diet	Ease of monitoring	Migration
Clapper rail	Local (<2 acres)	High	Sensitive to Hg	High for Hg	South, Central, North Bay	Tidal marsh	Crabs, clams, mussels, worms	Labor intensive	Resident
Snowy plover	Local	High	Unknown	Higher than you think	South Bay	Salt ponds?	Insects, worms, crustaceans, mollusks, fish	Easy	???
Least tern	Local+ Km	High	Unknown	Medium	Central and North Bay	Open Bay and Oakland Harbor	Crustaceans, insects, fish	Easy but individuals harder to follow – disturbance sensitive	South America?
Forster's tern	Global	Medium	PCBs known	High	South, Central, and North Bay	Ponds, Bay, and rivers	Fish, insects	Easy but individuals harder to follow – disturbance sensitive	Winter in southern California and Mexico
Double crested cormorant	Global	Low	Low to Hg, sensitive to PCBs and DDT	Medium to high	South, Central, San Pablo, Suisun	Open Bay	Fish	Easy on bridges (once logistics worked out) and some islands	Resident year round

Appendix I. Regional Board Management/Assessment Questions [1]

Overarching Question:
Are beneficial uses[2] (B.U.) adversely affected by contaminants[3] in San Francisco Bay[4]?

Evaluate how well the indicator could answer an assessment question under each beneficial use.

1. B.U. - Ocean, Commercial and Sport Fishing
   - 1.1. Do contaminants adversely affect:
     - 1.1.1. fish survival?
     - 1.1.2. reproduction, growth or other sub-lethal functions?
     - 1.1.3. fish prey?
     - 1.1.4. fish habitat?
     - 1.1.5. safe consumption of fish by humans?

2. B.U. - Shellfish Harvesting
   - 2.1. Do contaminants adversely affect:
     - 2.1.1. survival of critical life stages of shellfish?
     - 2.1.2. reproduction, growth, or other sub-lethal functions?
     - 2.1.3. shellfish prey?
     - 2.1.4. shellfish habitat?
     - 2.1.5. safe consumption of shellfish by humans?

3. B.U. - Fish Migration
   - 3.1. Do contaminants adversely alter migratory patterns of anadromous fish?

4. B.U. - Fish Spawning
   - 4.1. Do contaminants adversely affect:
     - 4.1.1. fish spawning behavior (e.g., herring)?
     - 4.1.2. fish spawning substrate (e.g., algae to which fish attach eggs)?
     - 4.1.3. condition of eggs?

5. B.U. - Estuarine/Marine Habitat
   - 5.1. Do contaminants adversely affect:
     - 5.1.1. primary productivity?
     - 5.1.2. energy transfer or nutrient re-cycling?
     - 5.1.3. resiliency of the Bay ecosystem?
       - 5.1.3.1. trophic structure?
       - 5.1.3.2. species abundance, diversity, or distribution?
     - 5.1.4. critical estuarine/marine habitats, such as mudflats, intertidal zones, eelgrass beds, and sloughs?

6. B.U. - Preservation of R & E Species
   - 6.1. Do contaminants adversely affect:

6.1.1. survival of R & E species?
6.1.2. reproduction, growth or other sub-lethal functions?
6.1.3. prey of R & E species?
6.1.4. habitat of R & E species?

7. B.U. - Wildlife Habitat
   7.1. Do contaminants adversely affect:
      7.1.1. survival of wildlife species?
      7.1.2. reproduction, growth or other-sub-lethal functions?
      7.1.3. wildlife food resources?
      7.1.4. wildlife habitat (e.g., wetlands)?

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[1] Assessment Questions:	Questions that express the environmental value being considered in an environmental analysis.
[2] Beneficial Uses:	Marine/estuarine ecological and human health B.U.s related to fish/shellfish consumption (not freshwater habitat, navigation, or industrial, agricultural and municipal supply, contact/non-contact recreation)
3Contaminants:	Trace metals, organics, exotic species (not bacteria, suspended sediment, nutrients, radioactivity, temperature, bad taste and odors, trash)
4SF Bay:	Includes marine/estuarine portions only. The Regional Board is developing a watershed monitoring program which, when implemented, will include freshwater tributaries to San Francisco Bay (Surface Water Ambient Monitoring Program – SWAMP).

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