additional details that were either conveyed during the workshop or provided by participants after it.

4.1. Information Exchange and Data Management

Task 1. Development of protocols for consolidating information from diverse areas of study, and implementation of sound database management practices

a. Comprehensive, Integrated Database: From the outset, it is important that a protocol be developed to consolidate information from diverse areas of study, and that sound database management practices be implemented for the entire research program. A single repository of information relevant to understanding the dynamics of the southern sea otter population is desirable but does not presently exist. Relevant research organizations need to reach an agreement for archiving and managing a southern sea otter database. The Otter Project will work with the relevant organizations toward the goal of identifying (a) the resources necessary to maintain a suitable database and (b) the party or parties willing to assume that responsibility. It is recognized that limited access to certain data sets is standard practice in the research community. Following the analysis of specific project data and the integrated data analysis described in task 10, and after an agreed time period, all data contributed to the database would be made publicly available via the worldwide web.

b. General Framework for Analysis: The essential challenge of the proposed research program is to build a causal web that links immunosuppressive and/or endocrine-disrupting chemicals with particular health outcomes, or endpoints. It is necessary that any conclusions drawn from the work have biological plausibility, which is the cornerstone of any epidemiological investigation. In the present instance, the biological plausibility is expected to derive from multiple approaches that should converge, assuming that the central hypothesis is true.

An analysis strategy to test the overall hypothesis of this research proposal is likely to involve the assimilation and analysis of data from most of the various tasks described below. The first phase in the general analysis plan will be an independent data analysis by the principal investigators for each task. Each of these independent analyses could lead to one or more manuscripts submitted for publication. However, the multi-level integration of the data to reach conclusions concerning the hypothesis should follow a cohesive, integrated analysis plan. To achieve such integration, an epidemiologic approach is envisaged (see Task 10, below).

c. Data Management and Quality Control: For integrated analysis, the data collected by each task would be submitted to a central repository. There, additional checks would be conducted for data quality, including identification of outliers and incompatible data points. Any additional data "clean-up" would

then be performed in collaboration with the laboratory submitting the file. Each data set would then be integrated into a larger data file with common features to facilitate data management.

d. Analysis Team: In order to accomplish the goal of integrated data analysis, a team approach is proposed. An epidemiologist would assume a coordinating role and employ a post-doctoral fellow to conduct the analyses (see Task 10, below). The analysis team would consist of the principal investigators from each of the tasks as well as the analysis group. This team would meet several times to discuss the analysis plan, deal with any problems that arise with data management, and provide additional input to the analysts at several stages of the work. They would also be responsible for making decisions about policy, for example with respect to publications and authorship.

Estimated cost: $100,000.

Task 2: Annual meetings of experts, and a workshop, to exchange ideas and findings concerning the extent to which toxic chemicals are compromising the ability of the southern sea otter population to recover

It is important that collaborating researchers meet yearly to share findings and coordinate further efforts. Also, prior to initiating Task 10 of the research program (see below), a workshop should be convened to allow researchers to: (1) evaluate progress and problems; (2) refine and elaborate upon the working hypothesis; and (3) outline a final synthesis of the program's results.

Estimated cost: $90,000

4.2 Characterization of the Southern Sea Otter's Environment

Task 3: Assessment of habitat quality for southern sea otters by reference to existing data.

This task would consist of a contract study to compile available information on contaminant distribution and levels in coastal waters from San Francisco Bay to Point Conception. It would draw upon Mussel Watch sediment data and Regional Water Quality Control Board data, as well as published literature, technical reports, and other relevant databases.

Estimated cost: 5 person/months @ $4,000 = $20,000.

Concern was expressed about the resolution of the data on mussel contamination. For example, it was considered likely that the PCB screening would not be congener-specific. Nevertheless, even if the data were to prove more superficial than desired, it was agreed that this task was a necessary first

step toward understanding the overall composition and distribution of contaminants in California coastal waters and identifying "hotspots."

Task 4: Investigation of the extent to which the habitat and prey of southern sea otters are contaminated.

With few exceptions, the environmental contaminants found in sea otter tissues come from their food. Therefore, an important facet of this study is to characterize the feeding ecology of southern sea otters and assess the extent to which their prey is contaminated. A representative sample of typical (invertebrate) prey items from California coastal waters where sea otters are known to forage will be collected and screened for contaminants known to pose risks to the health of wildlife (e.g., specific PCB congeners, dioxins, furans, organochlorine pesticides, organotins, and new flame retardants). These analyses will measure the concentrations of several hundred chemicals (or fewer, depending on budget and rationale), and form the basis for an ecosystem-based assessment of food quality. This information will support inferences about the following: a) the ecological pathways of contaminants in the southern sea otter's diet; b) the contaminants of greatest concern in their diet (based on comparisons with other studies); c) the bioaccumulation of persistent and toxic chemicals (some chemicals are very persistent, others can be metabolically eliminated); and d) likely sources of different contaminant classes. This last point is especially relevant to the design of appropriate mitigation strategies.

Estimated cost: $30,000. This could be much higher, depending on decisions concerning how many chemicals are to be included in the assays.

Estes re-emphasized during discussion of this task that (a) sea otters consume many different species, (b) individual otters forage in different ways, and (c) there is much geographic variation in foraging patterns. At least ten different prey species can be considered important to the overall plane of nutrition for the southern sea otter population. It was generally agreed that the goal should be to obtain ten specimens of each of ten preferred prey species from each of the three localities where sea otters are being live-captured for the study described in 3.6 (above). Sanders called attention to the practical difficulties (and associated costs) of collecting the desired sample of organisms from the specified areas. In view of those difficulties, the estimated cost assigned to this task may be too low.

There was considerable discussion of whether this task should proceed immediately or be deferred until results of one or more other tasks (e.g., Task 5) are available. Prey screening was viewed by most participants as something that should begin as soon as possible, on the understanding that it would inform other components of the overall research program, and in particular the surrogate species study (Task 9, below).

4.3. Assessment of Sea Otter Health

Task 5: Analysis of contaminant concentrations in existing tissue samples from beach-cast southern sea otters collected over the past ten years and for which complete necropsy and pathology reports are available (spanning periods of population growth and decline).

Exposure to high concentrations of environmental contaminants has been implicated in immunosuppression and other adverse health effects on wildlife. Organochlorines, including PCBs, PCDDs, PCDFs, DDTs, HCHs, and chlordanes; organotin compounds, such as butyl- and phenyltins; and heavy metals, such as mercury, cadmium, and lead, are the important classes of relatively well-known environmental pollutants. Elevated concentrations of organochlorines have been associated with reproductive or immunologic dysfunction in seals in the North, Baltic, and Wadden Seas (Helle et al. 1976; Hall et al. 1992; Heide-Jorgensen et al. 1992; Reijnders 1986), beluga whales in the St. Lawrence River (Martineau et al. 1987), striped dolphins in the Mediterranean Sea (Kannan et al. 1993; Aguilar and Borrell 1994), California sea lions in California (DeLong et al. 1973), and bottlenose dolphins along the U.S. Atlantic coast (Kuehl et al., 1994). Similarly, preliminary investigations with beach-cast southern sea otters found higher concentrations of organochlorine compounds, particularly PCBs and DDT, and organometals such as TBT, in animals that died of infectious disease than in those that were apparently healthy at the time of death (Kannan et al. 1998; Nakata et al. 1998).

These preliminary results point to a need to investigate the relationship between contaminant burdens and health status in a greater number of dead otters and to control for age, sex, and location-specific differences in contaminant concentrations. Although dead otters represent the failed individuals in the population (the "worst case scenario"), they also may prove key to identifying the causes of this population's slow and uneven recovery. Mortality has been pinpointed as the parameter most likely to be driving the population's observed leveling and decline. It is therefore crucial to maximize the amount of information on contaminant exposure obtained from fresh otter carcasses. The use of a large sample can help determine maximum tissue concentrations that occur in nature, distinguish key risk factors (e.g., age, sex, and geographic location), and complement live-animal studies. Salvaged carcasses provide a large resource for analyzing contaminant exposure retrospectively, without handling or sampling living otters.

Liver samples from more than 400 southern sea otters found freshly dead and necropsied since 1992 are currently available for analysis. These otters represent both sexes equally and were found across the entire range. Approximately 70% were "independent" animals (sufficiently old for contaminant accumulation) and 30% "dependent" (young individuals likely to

have fed in only one area). The approach will be to classify animals according to health status, based on post-mortem clinical investigations, and then to compare contaminant concentrations in liver tissues from animals in the different health-status categories, stratified by potential risk factors, body condition, and other factors expected to be related to contaminant burdens. Contaminants to be analyzed will include organochlorines, organotins, alkylphenols, and heavy metals, as most of these are known or suspected to be immunotoxicants.

Estimated cost: $200,000.

During discussion, Brand pursued the question of whether it would be possible to determine for animals that were trauma-killed, and thus possibly "healthy" at the time of death, whether or not they had previously been exposed to a particular pathogen. If this were possible, then the subsample of animals that had actually been exposed and successfully mounted an immune response to that pathogen would be the most appropriate control group in an effects study using dead animals. Frozen serum is available for most animals in the carcass database, and serological testing for antibody titers is ongoing. Serum screening is not possible for acanthocephalans; evidence of leptospirosis exposure disappears within 6-12 months; and there are difficulties with testing for some viruses. However, tests are in place for morbillivirus, brucella, coccidioidomycosis, toxoplasmosis, retrovirus, calicivirus, and various bacteria and protozoans. Skin tests should be considered to supplement the serological tests.

There was also discussion about whether it made sense to partition the carcass sample geographically, given what is known about the movements of individuals across the population's total range. Jessup pointed out that adult females are relatively sedentary, and that they are the group whose contaminant profile would most likely reflect that of a single geographic area.

Two issues were discussed at length but not finally resolved. One was the question of whether dead animals that are emaciated should be excluded from analyses because of the confounding effects of deteriorating body condition on tissue contaminant concentrations (see 3.3, above). The other was whether all contaminant assays should be high-resolution (e.g., congener-specific), or alternatively only a subsample should be screened at high resolution initially, with further screening dependent upon those results. This latter issue could have major cost implications.

Task 6: Disease and contaminant characterization of the live, free-ranging sea otter population, using blood and tissue samples collected during the currently funded USGS radio telemetry/tagging study.

The study described above in section 3.6 provides an important opportunity for collaborative research of various kinds. Two particular streams of activity were identified by the workshop. The first of these, concerning disease exposure and prevalence, is already being pursued to a large extent by the California Department of Fish and Game. An important proviso is that more attention should be given to indicators of successful immune response to antigen exposure.

The second main component is measurement of contaminant concentrations in blood and other tissues, especially liver. This component is addressed here, and the following elements were identified as relevant:

a. Blood: Collection of blood is already a routine procedure in the capture-release program. Needs envisaged in the present proposal would include 12 ml of whole blood in glass, to be used for high-resolution detection of PCBs, dioxins/furans, and organochlorine pesticides. The possibility of using a clot from the serum tube to supply whole blood should be investigated. Other needs would include 20 ml for heparinized plasma and PBMC, to be used, for example, in cytochrome P450 and immune function assays; and 18 ml of serum, to be used for protozoa and retrovirus assays. The total amount of blood needed would not exceed 50 ml, and it was recognized that the amount collected would necessarily be dictated at least in part by animal size and condition. Ideally, some blood would be archived for unforeseen retrospective analyses.

b. Liver: The collection of liver biopsies should be made routine at both capture and recapture, although it was realized that this would require modification of the existing USGS permit. Quantity was not regarded as an issue because the amount obtained would depend on the discretion of the attending veterinarian. Liver tissue would be used for histopathology (buffered formalin) and enzyme, contaminant, retinoid, and organochlorine analyses (snap-frozen). An important benefit is that liver tissue is not subject to temporal variation in constituents, as is blood.

Liver is a very important tissue for toxicological evaluation because: (a) many contaminants accumulate in this organ (organochlorines in liver lipid but also novel contaminants such as organotins); (b) it is the principal site in the body where detoxification of persistent organic pollutants takes place (measurement of the responsible "mixed function oxidase" enzymes is a useful index of this phenomenon); and (c) it is a "preferred tissue" for the measurement of several important health endpoints or "biomarkers" (e.g., Vitamin A, thymus hormones). Relevant measurements can be obtained using very small samples of liver tissue.

c. Skin: These are obtained routinely from flipper punches (for tagging). The quantity is fixed according to the size of the punch. As little as possible should

be preserved in formalin, and the rest snap-frozen (some is already committed to being stored in DMSO for genetic studies). Skin can be used to measure retinoids, CYP1A, and stable isotopes.

d. Whiskers: The wisdom of collecting a whisker should be evaluated in terms of both invasiveness (risk to the animal) and usefulness. If collected, whiskers should be stored at room temperature. Whiskers from Alaskan sea otters have been used in the past for stable isotope analyses.

e. Fat: This tissue is best obtained from the falciform ligament, the amount depending upon the discretion of the attending veterinarian. Such collection should be made routine but may require modification of the existing permit. 200 mg should be sufficient for all desired analyses, and this amount should be obtainable. Fat samples should be wrapped in foil and put in cryovials, to be used for high-resolution measurement of PCBs, organochlorines, and dioxins/furans. Fat tissue is preferable to blood for detecting and measuring lipophilic compounds.

Among the potentially "novel" aspects of this proposed task would be: (a) the development of "biomarkers" in blood, liver, and/or skin biopsies – for example, retinoids (vitamin A compounds, including storage forms), thyroid hormones (including thyroid stimulating hormone, TSH), and thymus hormones (as indicators of immune function); and (b) the use of fatty acid signatures and stable isotopes in blood, liver, and/or skin biopsies to characterize feeding ecology and trophic position.

Comparative approaches should be integral to the analyses and presentation of results from this task. Although there may be some inconsistencies in methodology or reporting between laboratories, the contaminant analyses that have already been conducted with sea otter samples from Washington and Alaska are available for comparisons. A discrete subtask that should be undertaken immediately is to produce an inventory of all available samples of sea otter tissues, from all agencies/institutions and all areas. This work would probably best done by someone who has been involved in some of the collections.

It may also be useful to explore the possibility of collaboration with researchers involved in live capturing and sampling sea otters in British Columbia, where the population currently numbers about 3000 and is growing rapidly.

Estimated cost: A full suite of contaminant analyses (including at least organochlorines, organotins, dioxins/furans, and heavy metals) for 150 animals @ $2500 = $375,000

Samples obtained from recaptured animals should be analyzed to some extent, but the nature and extent of analyses would depend upon what was learned from the first phase of analyses, and upon questions and hypotheses that arise.

Inventory of available samples: 1.5 person/months @ $4000 = $6000.

The total estimated cost for the work identified in this section was thought to be in the order of $500,000.

Concern was expressed as to whether the three capture sites adequately represent the spectrum of contaminant exposure levels experienced by the sea otter population. Ideally, one site would be highly contaminated, one medium, and one low. However, the ongoing USGS study was not designed with contaminants as a central focus, and the three sites were selected largely based on logistics and other concerns. Of the three, Piedras Blancas is relatively clean and Monterey relatively dirty. As mentioned earlier, the animals at Point Conception probably come from (and seasonally return to) various parts of the population's range.

This component of the overall research program is not necessarily hypothesis-driven, but rather exploratory and descriptive. For example, there may or may not prove to be inter- and intra-site variation in contaminant concentrations. Inter-site comparisons could prove interesting from a baseline point of view, and also could be expected to point toward important linkages. Many biological endpoints can be evaluated from very small samples.

Estes emphasized that the USGS project was in progress and the first year of captures and sampling had already been completed. He stressed the need for cooperation and coordination between the capture and sampling team and the team or teams involved in laboratory analyses. According to Sanders, although the U.S. Fish and Wildlife Service is required to bank tissues according to provisions of the Marine Mammal Protection Act, there is no FWS sea otter tissue bank at present. It was noted (by Sanders) that the volume of blood drawn from live-captured otters was fixed at a maximum of 60ml, and therefore it needs to be used efficiently. Any blood product or other tissue that is leftover after completing assays should be frozen (or otherwise appropriately preserved) and sent back to the source for archiving.

Task 7: Assessment of the immune system in free-ranging animals, including comparisons between locations with different contaminant compositions and concentrations

As described in 3.4 (above), functional genomics is a rapidly developing field, with new technologies becoming available on a frequent basis. Gene activation can be highly informative about an organism's exposure and response to xenobiotics. In the present instance, contaminant-sensitive genes need to be

identified in the sea otter. Specific immune-function tests are needed in addition to routine CBC (complete blood cell) serum chemistry.

A biomarker approach to immune assessment was proposed. A wide array of potential biomarkers could be used, and although assays for many of them are already available, screening methods for some biomarkers will need to be developed. Cytokine profiling offers a new, very sensitive method to detect allergic or immune responses.

a. Immunology: Many of the contaminants found in dead southern sea otters are known to be immunotoxic. Therefore, the occurrence of disease in this population may be at least partly due to a diminished ability of the animals' immune system to fend off pathogens. Assessment of the immune system will include traditional and newer molecular approaches. It will be developed using a tiered approach that allows the results of initial analyses (as well as sample availability) to inform and guide the selection of subsequent assays.

The first tier will be multifaceted and include three approaches to identifying toxin-associated physiologic/immunologic changes at both the molecular and cellular level. Studies will use white blood cells (leukocytes) from free-ranging animals living in environments with different levels of contaminant exposure. The specific analyses will include: i) gene microarrays to identify patterns of differential gene expression; ii) real-time polymerase chain reaction (PCR) to quantitatively measure cytokine gene expression; and iii) flow cytometry to assess perturbations in leukocyte phenotype and cell-surface expression of immunologically important proteins, such as adhesion proteins, leukocyte differentiation proteins, and major histocompatibility (MHC) molecules.

The second tier will consist of a more traditional functional assessment of the immune system and will include measures of cellular (lymphocyte) proliferation in response to well-characterized stimulants. The expansion of lymphocyte cell populations is a critical feature of the immune system's response to disease-causing organisms. Any compromise of this function, caused by chemical toxins, will affect an animal's potential to mount an effective defense against disease exposure.

The third tier will involve an effort to develop assays for concentrations of proinflammatory cytokines in serum, to be used as markers of chronic, low-grade infections that are typically not discernible from changes in hematological parameters. For example, detection of the cytokine interleukin-6 serves as a "biomarker" of early, as well as ongoing (chronic), host-defense responses. As described under the tier 1 assessment (above), it will be critical to compare the findings from all of these tests among subsets of animals living in environments that are subject to different levels of contaminant exposure.

Not included in this tiered approach is measurement of parameters associated with the functional properties of the innate immune system, *e.g.*, macrophages, neutrophils, and natural killer cells. Some idea of whether there are adequate numbers of these cells should be obtained from a complete blood cell count (CBC), which is an adjunct test required for flow cytometry (Tier 1). However, assays of these parameters require large volumes of sample (blood) and must be performed rapidly with fresh blood. Given the complexities of sample collection from the free-ranging population, it is not realistic to include the direct assessment of these parameters as part of this proposal. Many of the analyses described above for Tiers 1-3, however, can be performed on samples that have been properly prepared for long-term storage.

The approaches outlined above will help determine whether individual southern sea otters are immunocompromised. Correlations among contaminant levels, immune system function, immunogenetic haplotype (measure of immunologic vigor and pathogen pressure; see Task 8, below), and disease prevalence will help determine whether there are causal linkages.

b. Endocrinology: Many of the contaminants found in dead southern sea otters are "endocrine disrupting." Through this action, they can affect an animal's ability to mount an effective immune response to disease-causing organisms. Since endocrine systems are similar across species, much relevant scientific background information is available in the literature. For example, exposure to moderate levels of PCBs can disrupt hormone systems that have direct effects on immune function as well as growth and development and reproductive health. Several endpoints known to be sensitive to contaminant-related toxicity will be examined, including adrenal and thyroid hormones, thymus hormones, and vitamin A.

Access to samples is key to this task. Some biomarker assays of immune function require fresh cells. Without a firm commitment of adequate funding coupled with an administrative arrangement ensuring availability of samples, the task cannot proceed.

The present proposal presupposes that there will be a flow of fresh samples from live southern sea otters over the next 3-4 years. However, the USGS capture-release study headed by Estes (see section 3.6 and Task 6, above) is already in its second of three years. Therefore, there is uncertainty about sample availability at the appropriate time, and this factor needs to be taken into account in proposal development and funding decisions.

Ideally, this task would be a 3-year phased study, with two years of development followed by a third year of application.

Estimated Cost: $150,000/year for 3 years = $450,000.

4.4. Spatial and Temporal Comparisons

Task 8: Comparisons of immuno-genetic diversity both within the southern sea otter population (subsets living in environments with different levels of contaminant exposure) and between it and populations that are growing at a more rapid rate (e.g., SE Alaska and Washington)

All members of the current population of southern sea otters are descendants of a small group of animals that survived in the vicinity of Big Sur, discovered in the 1930s. Because of the small number of founders, MHC diversity must be somewhat limited in the current population.

a. Why study the sea otter MHC?: The molecules encoded by the genes of the major histocompatibility complex (MHC) play a vital role in determining an individual's susceptibility to infectious disease. MHC molecules link the non-specific and pathogen-specific arms of the immune system by presenting pathogen protein fragments (peptides) to T lymphocytes. Since certain MHC molecules bind certain pathogen peptides but not others, the spectrum of MHC proteins on the immune cell surface is closely associated with the effectiveness of immune response to a foreign microbe. While it is difficult to analyze MHC proteins directly, MHC molecular structure can be reliably derived from the MHC genotype (nucleotide sequence of a single gene). It follows that the MHC haplotype (nucleotide sequences of multiple MHC genes) affects the array of pathogens to which an individual can respond, and that MHC diversity and haplotype frequencies in a population are linked to infectious disease susceptibility. While the number and variety of MHC molecules present in a population are influenced by parasite and microbe pressures, they are also affected by other ecological factors. The relative contributions of these different factors can be calculated by genetic analysis. There is compelling evidence that environmental contaminants can influence MHC haplotype frequencies, both directly and indirectly. Numerous studies have shown that exposure to a range of toxins (e.g., dioxins, PAHs, and heavy metals) can selectively reduce expression of MHC genes from particular loci or alleles, and this can ultimately lead to immune dysfunction. If MHC genes have differential susceptibility to these contaminant-induced alterations in expression, the frequency of certain MHC haplotypes may differ as a function of contaminant exposure and change over time.

b. Can MHC genotyping be performed on sea otters? To date, there are no published MHC sequences for southern sea otters. Most studies of other marine mammals have been limited to examinations of partial-length genes. Since more information can be derived from full-length gene sequences, molecular characterization of full-length class I and II MHC genes is being completed for three other marine mammal species (California sea lion, Pacific harbor seal, and Hawaiian monk seal). The MHC-typing techniques used for these species in

the UC-Davis laboratory are readily adaptable for genotyping the sea otter MHC.

c. How will we examine the sea otter MHC? The first step will be molecular characterization of sea otter class I MHC genes (which recognize viral proteins) and class II MHC genes (which recognize bacterial and parasite proteins), using peripheral blood mononuclear cell-derived RNA from wild-caught sea otters. This information will be used to adapt the existing MHC-genotyping technique for use with this species, and to develop techniques for quantifying and comparing MHC gene expression between individuals. Following successful completion of this phase, we will be equipped to examine (i) MHC genotypes, (ii) haplotype frequencies, and (iii) MHC gene expression in our populations of interest. The utility of these studies will be optimized if samples can be collected from populations with a polar spectrum of contaminant burdens. Furthermore, a temporal comparison of MHC genotypes and haplotype frequency changes can be performed by examining archived samples collected during periods (recent and archaeological) with a broad range of contaminant exposures (quantitative and qualitative).

d. Potential information provided by MHC studies in sea otters: These studies will likely provide a range of useful insights concerning the relationship between contaminant loads and susceptibility to infectious disease in sea otters, including: (i) influences of population ecology (including contaminant exposure) on MHC diversity, (ii) current levels of immunogenetic diversity, (iii) inferences about susceptibility to infectious disease, (iv) effects of contaminant loads on MHC gene expression, and (v) increased understanding of the role of altered MHC expression in contaminant-associated immune dysfunction.

Estimated Cost: $150,000-200,000/year for two years = $300,000-400,000.

In discussion, Jessup reported that his laboratory has a grant from the Minerals Management Service to conduct a study of genetic diversity at the MHC and a few other loci, using DNA extracted from blood and other tissue samples obtained from beach-cast carcasses. Among the objectives of that study is to assess the MHC profiles of animals that died of particular diseases. It will be important to ensure against redundancy between the MMS-sponsored study and the proposed work at UC-Davis.

4.5. Surrogate Species Study

Task 9: Use of a surrogate or model species (the American mink) to assess the degree to which contaminants in the prey of southern sea otters may compromise their health

The American mink was proposed as a surrogate for the southern sea otter, to be used in an experimental study of the potential effects of various types and levels of contaminants. The main goal of these investigations would be to go beyond establishing correlations and associations, and to provide mechanistic insights concerning cause-and-effect relationships. The emphasis of this study would be on the mink's immune system although it was recognized that useful ancillary evidence could be obtained on the effects of contaminants on reproduction and other aspects of animal health. The American mink was chosen as a surrogate species for the sea otter for several reasons. It is in the same taxonomic family (Mustelidae) as the sea otter and readily available for experimentation. Moreover, there is a solid record of published data on the mink's physiological characteristics and sensitivity to a range of chemical exposures. The main goal of these experimental investigations would be to go beyond establishing correlations and associations, and to provide mechanistic insights concerning cause-and-effect relationships. The emphasis of this study would be on the mink's immune system, although it was recognized that useful ancillary evidence could be obtained on the effects of contaminants on reproduction and other aspects of animal health.

The proposed mink study might be designed to test the following hypothesis: Incorporation of toxicant-contaminated macroinvertebrates into the diet of ranch mink will have no significant effect on the immune competence of adult female mink, or on the survival, growth, or immune competence of their offspring.

It was expected that dosage and sampling strategies in the mink study would be closely coordinated with the investigators involved in Task 7 in particular, and that all laboratory analyses of samples from the mink would be carried out using procedures exactly matching those used for other components of the overall research program.

Elements of the methodology would include the following:

- Collect appropriate prey items of southern sea otters from areas along the central California coastline that are known to be contaminated with organochlorines and organotins
- Perform chemical analyses of prey items to determine contaminants and their concentrations
- Incorporate into a standard mink diet a chemical cocktail reflective of 0.5, 1.0, and 2.0 times the concentrations of contaminants measured in prey
- Perform chemical as well as proximate analyses on diets
- Twelve female mink per treatment group, thus 12 animals fed a clean standard ranch diet and three groups fed treatment diets containing, respectively, 0.5, 1.0, and 2.0 times the contaminant concentrations found in prey

- Trials to be initiated in early January
- Pre-exposure blood sample taken from each female mink for initial immune assessment
- Food consumption determined weekly, body weights determined monthly until breeding (1 March)
- Females bred to untreated males from 1 March to 21 March
- At whelping (15 April – 15 May), adult females will be weighed and kits counted, sexed, and weighed
- Adult females and kits will be weighed at 3 and 6 weeks past whelping
- Kits are weaned at 6 weeks of age
- Assess adult and kit livers for contaminant concentration
- Perform histological assessment of tissues
- Assess liver tissue for enzyme induction
- Periodically (monthly), collect blood from adults and kits for immune and health assessment
- Some kits may be exposed to disease agents ("challenged") to evaluate their susceptibility

Cost of basic mink reproduction trial:

Animals (48 females and 20 males)	$8,000
Food (13,000 lb @ $0.50)	6,300
[Cocktail?]	?
Animal care @ $25/day for 1 year	9,000
Half-time graduate assistantship	22,000
Research technician (25%)	12,000
Supplies	7,000
Histology	4,000
	$68,000

Acquisition of prey – uncosted

Chemical analyses - $500/sample → $70,000; congener-specific → $350,000

Immunological screening – uncosted (laboratory assessment of immune function – ca. $150,000/yr)

There was considerable discussion about how to handle the dietary component of this study. Estes stated that some 100 species had been identified as prey of southern sea otters, and that about 30 of these are fairly common in the diet. It was acknowledged that determining an "average" diet would be very difficult. After some discussion, it was agreed that supplying sufficient amounts of sea otter prey organisms from the central California coast to feed the captive mink would be impractical. Therefore, an alternative approach would be to use mussels collected from sea otter habitat in California as proxies for

the diet of free-ranging southern sea otters. Mussels have the advantage of being feasible to collect, and they are considered reasonably good integrators of an area's near-shore contaminants. In fact, it may prove necessary to use mussels and a few other prey items as the basis for creating a synthetic mink diet that will allow experimental exposures approximating those experienced by free-ranging southern sea otters.

Some concern was expressed about the appropriateness of using the mink as a model, or surrogate, for the sea otter in view of the fact that the female mink has a period of "rest" between pregnancy/lactation cycles. In contrast, the adult female sea otter is either pregnant or lactating almost continuously.

4.6. Integrated Analysis and Synthesis

Task 10: Epidemiological synthesis and analysis of data

As stated under Task 1, the overall research program's goal is an integrated, multivariate analysis of data from the various tasks, using an epidemiologic approach. Given the central hypothesis, the challenge will be to build a causal web linking immunosuppressive and/or endocrine-disrupting chemicals with particular health outcomes.

Data analysis will consist primarily of a cohort study of the free-ranging sea otter population (see Task 6, in particular). Contaminant data collected during the initial live-capture events will be analyzed for their ability to predict overall and disease-specific mortality, using a multivariate modeling approach (Cox proportional hazards) appropriate for the analysis of cohort data. Individual analyses will be conducted for each chemical and class of chemicals (e.g., individual PCB congeners, co-planar PCBs, total PCBs). Specific aggregations of causes of death (e.g., infectious diseases) will be explored while controlling for potentially confounding factors such as age, sex, and the effects of co-contaminants. In the next layer of analysis, the effects of specific contaminants on each of the health endpoints measured in the free-ranging population will be assessed (e.g., immune function, endocrine markers from Task 7), again controlling for potential confounding. In the third stage of analysis, the marker data will be integrated into an analysis of cause-specific and overall mortality, through modeling of interactions between specific contaminants and each marker, to evaluate the effect on overall and cause-specific mortality. Separate analyses will be conducted for animals sampled more than once prior to the end of the observation period.

In addition to the cohort study of free-ranging sea otters, additional analyses may be conducted that integrate data collected from the various tasks. These could include characterization of ecosystem-level interactions among pollutants, the effects of pollutants on population dynamics, and foodweb- or ecosystem-based models. For these types of ecological and population

analyses, the participation of an ecologist would likely be required. Ideally, one of the members of the research group would take the lead for these analyses.

Approximate Cost: $100,000

5. Conclusion

Upon completion of this work, it should be possible to conduct a credible risk assessment for southern sea otters, i.e., to evaluate the probability that a given animal is abnormally susceptible to disease as a consequence of exposure to environmental contaminants. The data and analyses that result from this initiative need to be applied to the ultimate goal of helping southern sea otters recover. In other words, the completion and publication of individual investigations must be complemented by an integrative effort to provide sound scientific advice as the basis for public policy. For example, decisions about how to manage sewage disposal, agricultural and industrial runoff, and non-point-source pollution may hinge on both the scientific credibility of the research and the quality and timeliness of the synthesis. It is important to reiterate and emphasize that the work proposed here is intended to supplement and complement the research already being pursued by the USGS and CDF&G.

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Appendix 1. List of participants

Reed Addis
United States Congressman Sam Farr
701 Ocean Street
Santa Cruz, CA 95060
831/429-1976
Reed.Addis@mail.house.gov

Christopher J. Brand
Branch Chief, Field and Laboratory Research
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608/270-2440
christopher_brand@usgs.gov

Steven Bursian
Professor of Animal Science
Michigan State University
Department of Animal Science
East Lansing, MI 48824
517/355-8415
bursian@msu.edu

Douglas DeMaster
Director, Alaska Fisheries Science Center (NMFS/NOAA/DOC)
7600 Sand Point Way NE
Seattle, WA 98115
206/526-4000
douglas.demaster@noaa.gov

James A. Estes
Biological Resources Division, U.S. Geological Survey
100 Schaffer Rd.
University of California , Santa Cruz, CA 95064
831/459-2820
jestes@cats.ucsc.edu

David A. Jessup
Senior Wildlife Veterinarian, Supervisor
CDFG-Marine Wildlife Veterinary Care and Research Center
1451 Shaffer Rd.
Santa Cruz, CA 95060
831/469-1719
djessup@ospr.dfg.ca.gov

Andrew Johnson
Program Manager
Monterey Bay Aquarium
886 Cannery Row
Monterey, CA 93940
831/648-7934
ajohnson@mbayaq.org

Kurunthachalam Kannan
Research Associate Professor
Michigan State University
213 National Food Safety and Toxicology Center
East Lansing, MI 48824
517/432-6321
kuruntha@msu.edu

Melissa A. Miller
Marine Wildlife Veterinary Care and Research Center
Department of Fish and Game
1451 Shaffer Rd.
Santa Cruz, CA 95060
831/469-1746
mchechow@cwo.com

F. Charles Mohr
Associate Professor
Department of Veterinary Pathology, Microbiology and Immunology
School of Veterinary Medicine
University of California
One Shields Avenue
Davis, CA 95616
530/752-7234
fcmohr@ucdavis.edu

Katherine Ralls
Senior Research Zoologist
National Zoological Park, Smithsonian Institution
2275 Niderer Road
Paso Robles, California, 93446
805/237-8215
rallsk@thegrid.net

Randall Reeves
Okapi Wildlife Associates
27 Chandler Lane
Hudson, Quebec, Canada J0P 1H0
450/458-7383
rrreeves@total.net

John S. Reif
Professor and Chairman
Department of Environmental Health
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, CO 80523
970/491-6074
John.Reif@colostate.edu

Peter S. Ross
Research Scientist
Institute of Ocean Sciences (Fisheries and Oceans Canada)
9860 West Saanich Rd, P.O. Box 6000
Sidney BC V8L 4B2, Canada
250/363-6806
rosspe@pac.dfo-mpo.gc.ca

Greg Sanders
United States Fish and Wildlife Service
Ventura Field Office
2493 Portola Road, Suite B
Ventura, CA 93003
805/644-1766
Greg_Sanders@r1.fws.gov

Julie Schwartz
University of California at Davis
Dept. of Patholgy, Microbiology, and Immunology
Laboratory for Marine Mammal Immunology
School of Veterinary Medicine
University of California at Davis
One Shields Avenue
Davis, CA, 95616-8739
530/752-7234
jaschwartz@ucdavis.edu

Steve Shimek
Executive Director
The Otter Project, Inc.
3098 Stewart Court
Marina, CA 93933
831/883-4159
exec@otterproject.org

Paul W. Snyder
Associate Professor of Toxicologic Pathology
Purdue University
School of Veterinary Medicine
Department of Veterinary Pathobiology
1243 VPTH Bldg
West Lafayette, IN 47907-1243
765/494-9676
pws@vet.purdue.edu

Jeffrey L. Stott
Professor of Immunology
Laboratory for Marine Mammal Immunology
Department of Pathology, Microbiology & Immunology
School of Veterinary Medicine
University of California
Davis, CA 95616
530/752-2543
jlstott@ucdavis.edu

Nancy J. Thomas
U.S. Geological Survey, National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711
608/270-2463
nancy_thomas@usgs.gov

The Otter Project – Organizational Background

Mission

The Otter Project exists to promote the rapid recovery of the California sea otter, an indicator of near shore ocean health, by facilitating research and communicating research results to the general public and policy makers.

Vision

Our vision is of a healthy sea otter population, a productive, diverse and healthy marine ecosystem, and sustainable fisheries along the California coast.

Board

John Pearse, Ph.D., President, California Academy of Sciences, Professor Emeritus, Institute of Marine Sciences, UCSC

Ms. Carolyn O'Donnell, Executive Director, Santa Cruz Area TMA

Mr. Andy Johnson, Program Manager, Sea Otter Research and Conservation, Monterey Bay Aquarium

Mr. Steve Shimek

Ms. Doreen McElvany

Dave Ebert, President, US Abalone

William Douros

All Board Members serve as individuals and do not represent any group or agency.

Membership
June 1999 – 0
June 2002 – 2537 (contributing) plus ~65 volunteers
Membership is national, with members in 47 states and Washington DC

Foundation Supporters
David and Lucile Packard Foundation
Richard and Rhoda Goldman Fund
National Fish and Wildlife Foundation
World Wildlife Fund
Fund for Santa Barbara
UC Santa Barbara Shoreline Preservation Fund
Community Foundation of Monterey County
Environmental Support Center

The Otter Project – Major Accomplishments

1999. Study of live-trap fishery along California coast including literature search of sea otter / trap mortality reports, experimental trials with captive sea otters and traps, field observations of fishery. Results: Research report leading to California Fish and Game Commission directing Department to enact rules.

1999. Study of captive sea otter life expectancy as requested by Southern Sea Otter Recovery Team. Results: Paper in Marine Mammal Science. Inclusion of results in Southern Sea Otter Recovery Plan.

2000. Media, political agencies, conservation groups, and general public education campaign in Santa Barbara County.

2000. Necropsy database plan to consolidate 15 years of necropsy data held at two competing public agency labs. Program included pathologist exchange, post-doc support, and creation of a unified database. Results: Standardized protocols for sea otter necropsies and unified database.

2000 - ongoing. Provided major support to Monterey Bay National Marine Sanctuary "Team Ocean". Provided $15,000 in kayaks and gear to support on-the-water education and monitoring program. Results: Four "Team Ocean" Sanctuary employees are in kayaks and talking with visitors about wildlife viewing etiquette for four months every summer.

2001. Continuation of necropsy database program.

2001. Observer effort at Cojo Anchorage, immediately south of Point Conception, to deter malicious take of sea otters and to provide logistical support to research. Results: No malicious deaths were discovered during the effort. Project documented sea otter movements in Cojo Anchorage area.

2001. BeachCOMBER surveys started in Santa Barbara County. Beginning of standardized samples of dead marine birds and mammal on Santa Barbara County beaches in an effort to determine baseline levels against which we can recognize unusual events. Results: Nine months of surveys completed to date.

2002. Contaminants Working Group Meeting. Four day meeting of agencies and researchers to create a collaborative research approach around chemical contaminants and disease in sea otters. Results: Research outline, proposal, and workshop report.