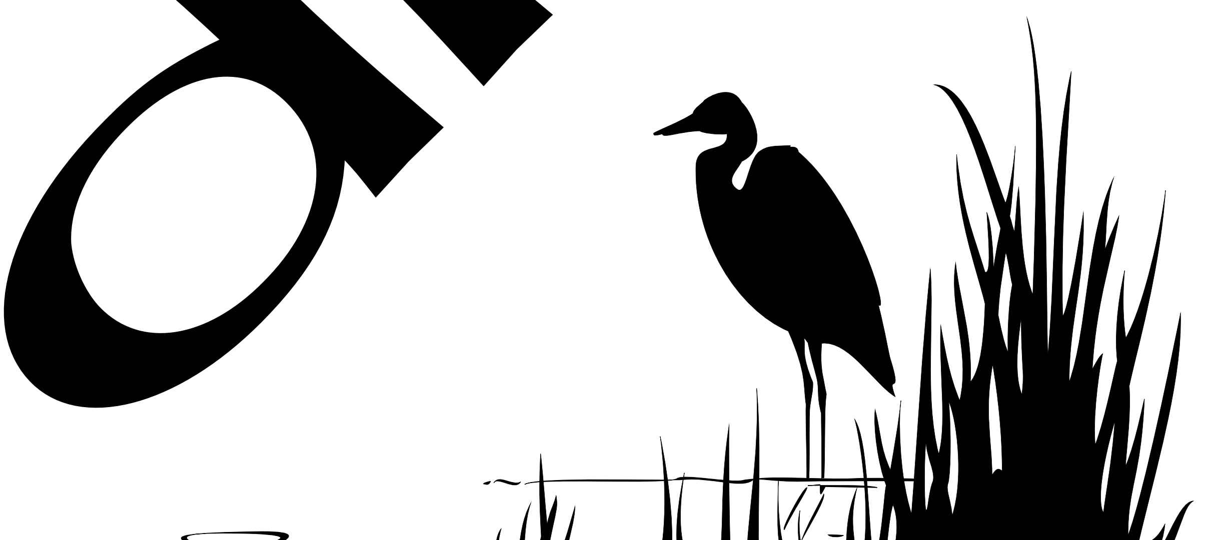
Biological Effects of Selenium and other Contaminants
Associated with Irrigation Drainage in the Salton Sea Are[illegible] Calif[illegible]nia,
1992-94.

Studies Performed for the Remedia[illegible]n Phas[illegible](IV) of the
National Irrigation Water Q[illegible] [illegible]ogram,
Department of the In[illegible]

Report prepared by:
Jewel [illegible]
Division of Envir[illegible] [illegible]minants
U.S. Fish [illegible]nd Wild[illegible]
[illegible]arlsbad, CA

M[illegible] 1997



Acknowledgments

Many researchers were involved in the planning and performance of the studies described in this report. Mick Rivera, Carol Roberts, Leonard LeCaptain, Daniel Audet, William Radke; Suzanne Audet and Mary Hunnicutt, of the U.S. Fish and Wildlife Service (USFWS) all participated in study designs, and data collection and summarization. Patty Wilson and [illegible] Remington, of the Imperial Irrigation District, and Sharon Keeney, of California Department of Fish and Game, provided much assistance and cooperation in sample collection for the [illegible] study. Dr. David Hoffman, of the National Biological Service, provided expertise in embry[illegible], and Dr. Joseph Skorupa, USFWS, provided a great deal [illegible]ance and technical advice in data interpretation. Jim Setmire and Roy Schroeder, of U.S. Geological Survey, and John Johnson, of Bureau of Reclamation, provided valuable comments on earlier [illegible] this report. Carol Roberts finalized the report, and Beth Burroughs [illegible] assistance [illegible]ocument preparation, including tables and figures.

Executive Summary

The National Irrigation Water Quality Program (NIWQP) investigated contaminants associated with drainwater in the Salton Sea area from 1986-1990 (Setmire et al. 1990, Schroeder and Rivera 1993, and Setmire et al. 1993) and concluded that Department of the Interior (DOI) trust resources were adversely at risk to contaminants, especially reduced re[illegible]ve capacity due to elevated levels of selenium, organochlorine pesticides an[illegible]ossibly bor[illegible]alton Sea environment. The Salton Sea project advanced to Phas[illegible]V, reme[illegible]ation plann[illegible] NIWQP. Typically, in that phase of work remediation p[illegible] planned, and/or additional data collection is conducted if needed. In the case of t[illegible] Salto[illegible]ect, fairly extensive additional data collection was conducted during [illegible] to clarify [illegible]ological effects of the environmental contaminants documented [illegible] occur in [illegible] investigations.

The Salton Sea area is located w[illegible]n a very [illegible]portant a[illegible]ricultural region that includes the Coachella [illegible] Imperial V[illegible]hich curr[illegible]port a one billion dollar agricultural industry (Steve Kne[illegible]al Irrigat[illegible]ct, personal communication). Because approximately 97% of California s w[illegible]ve been [illegible]erted to other uses, fish and wildlife resources now heavily [illegible]ilize agricultu[illegible]ays as alternative habitat. Therefore, agricultural drains in the Co[illegible]ella and Imperial [illegible]lleys and the Salton Sea are also important fish and wildlife habitat. I[illegible]dition to its imp[illegible]ance as the single major inland nesting and wintering area for hundreds [illegible]s, it also supports habitat for four endangered species and is the site of both a federal and state wildlife refuge.

This report describes the biological effects of environmental contaminants on several important fish and wildlife species in the Salton Sea area that were investigated in 1992-94. Three studies were conducted to obtain information about species either thought to be at particular risk, or those for whom the effects of selenium and DDE are fairly well understood. The [illegible] 1) determined embryotoxicity of colonial waterbirds (fish-eaters); 2) [illegible] embryotoxicity and nesting proficiency of black-necked stilts (shoreline [illegible]); and 3) determined contaminant body burdens of sailfin mollies [illegible] species for [illegible] desert pupfish that inhabits agricultural drains. The [illegible]-offs which have occurred at the Salton Sea are being investigated separately [illegible] of the [illegible] corporate disease and biotoxicity studies in addition to contaminants [illegible]

The findings indicated 1) colonial [illegible] water [illegible] (great egrets, snowy egrets, and black-crowned night herons) are at risk [illegible] from DDE-induced eggshell thinning and, to a [illegible] extent, [illegible] mortality; in addition, egret embryos displayed a high [illegible] (possibly over 100 times normal) of unknown causes; 2) 95% of black-necked [illegible] they came from nests with a four-fold increased risk of nest [illegible] due to [illegible] embryo mortality, and that stilts are experiencing a 4.5% [illegible] in nesting [illegible] due to selenium exposure in the Salton Sea area; and 3) the [illegible] and state [illegible] desert pupfish inhabiting agricultural drains are at risk of [illegible] due to selenium concentration in the adult fishes sampled.

Management implications are that the biological effects of selenium in the Salton Sea area are more clearly understood and it presents reproductive depression in black-necked stilts, hazard to reproduction of the desert pupfish, and selenium levels in fish make them hazardous food items for fish-eating birds. The issue of selenium contamination presenting a reproductive hazard to the federally endangered pupfish is a situation likely to require a Sectio[illegible] consultation under the Endangered Species Act. The apparent high in[illegible]ence of egre[illegible]bnormalities would require additional verification to identify manage[illegible]ent opti[illegible]s.

In general, the biological tissue information indicates s[illegible]lenium [illegible]resholds are consistently approached and in many cases exceeded, dependi[illegible]pecies sa[illegible] and the sampling location. There is a need to conduct reme[illegible]ation pla[illegible]ill [illegible]cus on reducing the amount of selenium available via food chain[illegible] that bio[illegible]gical tiss[illegible]e concentrations are eventually below hazard thresholds. The se[illegible]ium con[illegible]trations [illegible]tected indicate that a relatively small change in b[illegible]gical tissu[illegible]nination [illegible]stantially reduce toxic hazard. Remediation planning s[illegible]ally focu[illegible] to manage selenium in drainwater to reduce biological hazards. Reduc[illegible]d input[illegible]latively high amounts of selenium, such as certain subsurfa[illegible]e drains, coul[illegible] efficient approach. It is necessary to determine if those efforts wo[illegible] reduce the overal[illegible]elenium risks to fish and wildlife populations. A management goal of l[illegible]g water-borne [illegible]lenium in drainwater to <10 ppb has been recommended by other [illegible]vent most avian toxicity, although not avian contamination. Appropriate remediation goals for the Salton Sea area should be developed using information about potential exposure to selenium by wildlife using the variety of water sources in the Salton Sea area, including canals, drains, and the Sea.

In the case of DDE, wildlife hazards could be reduced by reducing or eliminating any continuing inputs to the aquatic environment from historically contaminated fields and drains, and if possible, limiting the availability of DDE in aquatic food chains. It is desirable to determine if the high concentrations of DDE observed in some bird eggs are contributing to the fish-eating bird nesting decline and the unusual observation of embryonic twinning (multiplicity) observed in this study.

Introduction

The Fish and Wildlife Service is concerned that contaminants in the Salton Sea area present a risk to fish and wildlife resources. The area is a critical component of the Pacific Flyway with over 270 species of birds utilizing the Imperial Valley as either permanent residents or seasonal migrants. The area also contains habitats of several endangered species including the Yuma clapper rail (*Rallus longirostris yumanenis*), brown pelican (*Pelecanus occidentalis*), peregrine falcon (*Falco peregrinus*), and the desert pupfish (*Cyprinodon macularius*). There are also several wildlife management areas in the Imperial Valley including the U.S. Fish and Wildlife Service [illegible] Salton Sea National Wildlife Refuge (SSNWR) and the California Department of Fish and Game (CDFG) Imperial Wildlife Management Area.

The NIWQP investigated contaminants associated with drainwater in the Salton Sea area of California in 1986-1990 (Setmire et al. 1990, Schroeder and Rivera 1993, and Setmire et al. 1993). Those studies found irrigation drainwater from various locations in the Imperial Valley contained from 3 to 360 ug/L selenium, with higher values typically found in subs[illegible]ace drains. The federal water quality criteria for protection of aquatic life is 5 ug/L [illegible] 1987a). Biological sampling in that study found that drainwater c[illegible]aminants in[illegible]lenium, boro[illegible] and DDE were accumulating in tissues of migratory an[illegible]sident b[illegible]ds that use [illegible]es in the Imperial Valley and the Salton Sea. Selenium conce[illegible]in fish-eating birds, shorebirds and the endangered Yuma clapper rail were at levels th[illegible]t could [illegible]roduction. That report (Setmire et al. 1993) also concluded that waterfo[illegible]h-eating b[illegible]ay also be experiencing reproductive impairment as a[illegible]esult of [illegible]ion in food resources. DDE concentrations measured in biota in th[illegible] study a[illegible] some of [illegible]e highest values documented in California. These NIWQP studi[illegible]learly d[illegible]mented [illegible]t certain drainwater contaminants were elevated in[illegible]Imperial V[illegible]d Salton [illegible]ronment, however, the studies had not been designed t[illegible] the asso[illegible]logical impacts. Therefore, the Salton Sea NIWQP Study Team decided t[illegible]dditiona[illegible]ies to collect information describing biological effects associat[illegible] with this co[illegible] in order to determine the need for remediation planning.

S[illegible]al other studies [illegible]ve indicated persistent contaminants that bioaccumulate are at levels in t[illegible]at could reduce resource productivity in the Salton Sea area. For example, a recent study of the endangered Yuma clapper rail (Roberts 1996) found that eggs collected from the CDFG Wister Wildlife Management Unit when drainwater was being used as the water source for wetlands had maximum selenium concentrations of 7.8 ug/g dry weight (DW), an amount considered hazardous to reproduction as described in detail below.

In addition to selenium, organochlorine compounds are a persistent co[illegible] problem for the area s wildlife. Exceptionally high concentrations of DD[illegible]ere found [illegible]owned night-heron (*Nycticorax nycticorax*) and great egret eggs coll[illegible]ed from [illegible]e Salton Se[illegible] (Ohlendorf and Marois 1990). The geometric mean DD[illegible]ration in black-crowned night-heron eggs was 8.62 ppm wet weight (WW), and was [illegible].0 ppm [illegible] great egrets. The reproductive effects of DDE in night herons bec[illegible]ificant at r[illegible] levels in the eggs of about 8 ppm WW, with effects including e[illegible]gshell th[illegible]kage, and reduced clutch size, hatching success and subsequen[illegible]oductiv[illegible] (Custer [illegible] al. 1983, and Henny et al. 1984). Although Ohlendorf and Marois [illegible]90) did [illegible] determi[illegible] reproductive success in their study, based upon [illegible]osure-resp[illegible]ationship[illegible]or DDE they concluded that heron and egret reproductio[illegible]alton Se[illegible]lmost certainly be impaired.

A drama[illegible]c decline in c[illegible]terbird nesting productivity was documented at Salton Sea nest site[illegible]ince 1986 (USFW[illegible]1991 and 1992, and Audet et al. 1997). The total number of nesting c[illegible]ial birds at Salt[illegible] Sea shoreline colonies declined from 1,950 in 1987 to 231 in 1989, with p[illegible]ons in productive nests observed for great blue herons (*Ardea herodias*), cattle egret (*Bubulcus ibis*), snowy egret (*Egretta thula*) and great egret (*Casmerodius albus*). Double crested cormorants (*Phalacrocorax auritus*) and white pelicans (*Pelecanus occidentalis*) apparently no longer attempt to nest at the Sea. Other species of colonial waterbirds, such as the black skimmer (*Rynchops niger*) and gull-billed tern (*Sterna nilotica*) have had relatively stable numbers of active nests, but have experienced low reproductive success for several years (USFWS 1992, USFWS 1994). It is unknown to what extent contaminant-induced reproductive failure has contributed to the decline in nesting success of fish-eating birds at the Salton Sea shoreline colonies.

There is also concern about the influence of contaminants on fish resources in the Salton Sea. Four species of recreational fish from the Salton Sea were studied in 1985 to determine if certain elemental contaminants were accumulating to unacceptable levels in the fishery (Saiki 1990). In that study whole bodies of bairdiella (*Bairdiella icistia*), orangemouth corvina (*Cynoscion xanthulus*), sargo (*Anisotremus davidsoni*) and Mozambique tilapia (*Tilapia mossambica*) contained average selenium residues of 10.3, 11, 7.05 and 8.7 ppm DW, respectively. Selenium toxicity thresholds for saltwater fish are not known (White et al. 1987), but levels of 4 ppm selenium in whole bodies of freshwater fish are known to cause adverse effects (Lemly 1993). Saiki concluded the excessive accumulations of selenium could seriously affect the fishes and sport fishery of the Salton Sea. In addition, the selenium levels observed in those fish greatly exceed the 3 ppm DW dietary toxicity threshold for other wildlife (Lemly 1993), meaning they should be viewed as toxic to other fish and aquatic birds that consume them.

Project Objectives

This project was designed to more clearly define the biological effects of contaminants associated with drainwater on fish and wildlife resources in the Salton Sea area. Three studies were conducted in 1992-94 to obtain information about species thought to be at particular risk, and for those whom the effects of selenium and DDE (in particular) are [illegible] well understood. The three studies and their objectives were:

Study 1. Determine the embryotoxicity of colonia[illegible]irds nesting at the Salton Sea. As fish-eating birds, black-crowned night hero[illegible], and s[illegible] great egrets were found to have amongst the highest concentratio[illegible]minants th[illegible]oaccumulate including selenium, boron, and organochlori[illegible] pesticid[illegible] ([illegible] 1993). In this study those species were examined for eg[illegible]ell thin[illegible]g, embry[illegible] deformities, and egg contamination.

Stu[illegible] Determi[illegible]sting pro[illegible]and embryotoxicity of black-necked stilts, a resi[illegible]ies at the [illegible]ea. Most stilt eggs sampled in the NIWQP Detailed Study containe[illegible]5 ppm se[illegible]um, meaning those eggs were at an increased risk of [illegible]bryonic deat[illegible]ibly embryonic deformity (Setmire et al. 1993). In this study, evaluations woul[illegible] be made of black-necked stilt nest proficiency, embryotoxicity, and egg contamin[illegible]on.

Study 3. Determine the body burdens of contaminants in a surrogate species for the endangered desert pupfish. This issue was not previously investigated, but was a concern because the pupfish inhabit drains that convey various amounts of contaminants in drainwater to the Salton Sea.

Guidelines for Evaluating Biological Risk from Selenium Exposure

A large amount of information has been generated recently on the biolo[illegible]zards of selenium to fish and wildlife resources, and general toxicity guideli[illegible] have been [illegible] by several researchers. Also, a working group of the Kendrick Pr[illegible]ct and [illegible]iddle Green [illegible] Interdisciplinary teams of the NIWQP synthesized the sc[illegible]formation regarding effect thresholds of selenium in various environmental comp[illegible]tments. [illegible]ult, general predictive thresholds for biological hazards have been deve[illegible]use in haz[illegible]sessment relative to selenium (BOR 1993). These thresholds [illegible] summa[illegible]. A summary of the recent literature regarding selenium hazards [illegible] fish and wildlife re[illegible]urces is presented below to provide background for the BOR (1993) [illegible]delines, [illegible] to aid i[illegible]he interpretation of results from this study.

<u>Selenium in the [illegible]nt</u>

Freshw[illegible]er ecosystems [illegible]ot heavily influenced by agriculturally or industrially mobilized sou[illegible]s of selenium usu[illegible]y contain less than 0.5 ppb total recoverable selenium in the water. I[illegible]d saline sinks su[illegible]eyed in Oregon, California, Nevada, and Utah typically contained less t[illegible]ecoverable selenium. Chemically, selenium behaves similarly to sulfur and therefore is biologically active in that it can enter into the metabolism of plants and animals. Aquatic plant and insect tissues in selenium-normal environments usually contain on average less than 2 ppm DW total recoverable selenium. Bird's eggs, one of the best wildlife tissues for monitoring exposure to selenium, usually have concentrations that average less than 3 ppm DW. Whole body concentrations in freshwater fishes are usually less than 2 ppm DW. Plants take up selenium from water and concentrate it in their tissues to varying degrees depending upon the species of plant. Fish and wildlife bioaccumulate selenium in their tissues principally by eating contaminated plants, insects, and other animals. Selenium has a unique [illegible] separation between dietary exposures that are nutritionally beneficial and those that are toxic [illegible] 1980, Hodson and Hilton 1983). Experiments with less than [illegible] seleno-amino acids in the water have led to tissue concentrations of selenium in aquatic insects [illegible]ld be toxic to fish and wildlife eating those insects. As a very general [illegible] environmental concentrations just 10-times or more above normal background concentrations are [illegible] to cause biological hazards (Skorupa 1994).

<u>Selenium R[illegible] to Birds</u>

Avian embryos are very sensitive to the toxic effects of selenium (Ohlendorf 1989). Hatchability of fertile eggs is [illegible]d the most sensitive measure of selenium toxicity. The hatchability of chicken eggs is reduced [illegible] concentrations of selenium are 6 to 9 ppm in the egg. Fertility is not affected, but excess selenium causes unusually high rates of embryo mortality and developmental abnormalities (teratogenicity) in birds. Malformations of embryos caused by s[illegible]ly result from tissue necrosis of the brain and spinal cord, optic cups and lens vesicles, mesenchyme of the limb buds, and sometimes the tail region (Gruenwald 1958). These malformations include microphthalmia, anomalies of the extremities, and microcephaly.

Extensive field studies of avian response to elevated environmental selenium have been conducted in the San Joaquin River Basin and near the Tulare Lakebed in California, and, to a lesser degree, in other regions of the western United States. These studies have provided a large data base for classic dose-response and epidemiological statistical analyses of avian response to selenium exposure that have proven to be taxonomically and geographically robust (Skorupa et al. 1992, and USFWS 1995). It has been determined, on a bird population basis, that when the mean selenium concentration in the eggs are below 3 ppm DW there is a low risk of reproductive failure due to selenium. When bird egg concentrations are above 20 ppm selenium DW, the population is considered to be at high risk to selenium toxicity and is essentially certain to experience reproductive failure. Therefore, the low and high risk ends of the toxicity response for birds, with respect to reproductive performance, is [illegible] defined and is relatively narrow. When egg selenium concentrations fall within 3-20 ppm DW that bird population is at some intermediate amount of risk and effect studies of reproductive performance are generally necessary for more precise risk assessments ([illegible] et al. 1996). Those sorts of assessments have specifically determined [illegible] with black-necked stilt eggs containing as little as 4.2-9.7 ppm selenium DW are about four times more likely to be reproductively impaired than nests with sample eggs containing [illegible]2 ppm selenium (Ohlendorf et al. 1993, Skorupa 1994).

Selenium Risk to Fish

[illegible] provided guidelines for evaluating the risk posed to fish by selenium in aquatic environments. Biological effect thresholds for the health and reproductive success of freshwater and anadromous fish are 4 ppm DW selenium for whole body, 8 ppm for skeletal muscle, 12 ppm for liver, and 10 ppm for ovaries and eggs. Further, the chronic dietary selenium threshold for fish and wildlife is considered 3 ppm DW, food organisms containing that level of selenium would supply a toxic dose of selenium while being unaffected themselves. Taxa of fish differ in their sensitivity to selenium, with cold water fishes, such as salmonids, more sensitive than warmwater fishes. In laboratory studies, rainbow trout fry (*Oncorhync*[illegible]*ss*) experienced significant mortality when whole-body residues exceeded [illegible] ppm DW ([illegible] 1987).

Juvenile rainbow trout exposed to waterborne and diet[illegible]y sodi[illegible]te exhibited significant changes in blood chemistry when whole-body ti[illegible]es reache[illegible]t 3 ppm; survival was reduced when whole body residues reache[illegible]5 ppm ([illegible]0, and Hilton et al. 1980). Hamilton et al. (1986, 1989, and 199[illegible]) studied j[illegible]venile chi[illegible]ook salmon (*Oncorhynchus tshawytscha*) exposed to waterbo[illegible] and die[illegible]y seleni[illegible] and observed growth was impaired at whole body [illegible]idue levels [illegible] 2 to 3 p[illegible]ortality occurred when concentrations exceeded 1[illegible]

Lemly ([illegible]93) studied t[illegible]nic effects of selenium in natural populations of centrarchids and [illegible]her warm-water fi[illegible] species. Whole-body selenium concentrations of 15 ppm were a[illegible]iated with a 10-f[illegible]d higher incidence of defects in centrarchid populations in a [illegible]. The relationship between tissue selenium residues and the prevalence of malformations approximated an exponential function over the range of 1-80 ppm selenium and 0-70% deformities (R^2=0.881, P<0.01). Lemly concluded that this relationship could be used to predict the role of teratogenic defects in warm-water fish populations suspected of having selenium-reproductive failure. Hermanutz et al. (1992) observed pronounced mortality and deformities in larval bluegill (*Lepomis macrochirus*) when breeding adults were experimentally exposed to 10 ug/L selenium (as sodium selenite) in the water for 40 weeks prior t[illegible]gg-laying. Statistically significant differences (*p* 0.05) between the control and 1[illegible]eatment were observed in mortality (69.7 and 28.8 percent larvae survi[illegible], respectiv[illegible] with edema or body swelling (0.1 and 80.0 percent, respectively), la[illegible]ae with [illegible]emorrhagin[illegible].5 percent, respectively), and larvae with lordosis or crooke[illegible].8 and 11.6 percent, respectively, *p* 0.10). The whole body residues of se[illegible]nium [illegible]uegills at the end of 365 days in the 10 ug/L water averaged 4.6 ppm wet [illegible]pproximat[illegible].4 ppm converted to a DW basis).

Juvenile fathead minnows (*Pime[illegible]les pro[illegible]as*) had [illegible]wth inhibition at whole body tissue levels of 6 t[illegible] ppm DW [illegible] and abo[illegible]ett et al. 1986, and Ogle ad Knight 1989). In outdoor ex[illegible]l streams [illegible]ith sodium selenite, Hermanutz et al. (1992) observed that reproductive su[illegible]head mi[illegible]s (ability of fry to swim-up) was impaired when the ovarian [illegible]ssue of spawn[illegible]s contained about 15 ppm and resultant fry contained about 8 pp[illegible]W selenium on a [illegible]hole body basis. A significant (*p* 0.05) increase in edema and l[illegible]is was also obs[illegible]ved in larval fathead minnows when adults were exposed to 10 ug/L s[illegible]ater (Schultz and Hermanutz 1990). Control and selenium-exposed fathead minnow larvae had a 0.9 and 24.6 percent occurrence of edema, respectively, and a 5.6 and 23.4 percent occurrence of lordosis, respectively. Selenium residues in the fathead minnow embryos spawned in the selenium-treated stream averaged 3.9 ppm wet weight (about 15.64 ppm when converted to dry weight, assuming 80% moisture). Depending upon the specific tissue sampled, concentrations of selenium in fish from control groups or habitats with low ambient selenium levels usually ranged from 1 to 8 ppm (whole body would have lower levels than liver or ovary tissues) (Lemly 1993). However, tissue damage in major organs, repro[illegible] impairment, and mortality were observed when levels reached 4 to 16 ppm. This extrem[illegible] margin between "normal" background and toxic levels , along with the propensity of se[illegible] bioaccumulate in aquatic food chains, underscores the bi[illegible] importance of even slight increases in environmental selenium.

Lemly (1993) recommended that 4 ppm selenium in [illegible] ppm in skeletal muscle, 10 ppm in ovary and eggs, and 12 ppm in liver be considered toxic effect thresholds for the overall health and reproductive vigor of [illegible]shwater [illegible] andron[illegible]ous fish. Laboratory and field studies indicate th[illegible]productive [illegible] is the m[illegible]ve indicator of selenium impacts on both fathead mi[illegible]on and [illegible] 1989, Schultz and Hermanutz 1990) and centrarchid populations (Cu[illegible] Van Hor[illegible]8, Gillespie and Baumann 1986, Woock et al. 1987, Herman[illegible]z et al. 1992 a[illegible] et al. 1993).

Study Area and Methods

[illegible] Waterbird Eggshell Thickness and Embryotoxicity

Egg collections for this study in 1992 and 1993 focused on nesting colonies of black-crowned night-herons, and great and snowy egrets that have historically been located at both the north and south ends of the Salton Sea. The colonies were assigned names based on nearby roads and waterways and are indicated in Figure 1. Most nest sites were on snags, dikes or islands surrounded by 0.1 to 0.2 meters of water and were approached by kayak or small boat with an outboard motor. Eggs were placed in an egg carton and chilled on ice until returned to the SSNWR where they were refrigerated until transfer to the Carlsbad Field Office of the USFWS for processing.

Study 2. Black-Necked Stilt Nesting Proficiency

The study area for the black-necked stilt nesting study was five [illegible] around the Salton Sea (Figure 1). The Johnson Drain site consisted of [illegible] land east of [illegible]ewater River Delta near where Johnson Drain empties into the Sea. The [illegible] site is a detached levee that functioned as a small island in the southwest portion of the Sea. The Davis Road site consisted of plowed road sides along the main road that runs north-south through the Wister Unit of the Imperial Wildlife Area, administered by C[illegible] The Hazard Tract site is on land leased from CDFG and [illegible] by SSN[illegible] site included nesting areas in the dry pond bed of the Hazard 1 Unit a[illegible] earthen [illegible] the north end of the Hazard 10 pond. The Garst Road Barnacle Bar nesting si[illegible]ng the south dike of a small pond adjacent to Garst Road and immediately west of Hazard 4 pond (north of the Elmore Geothermal plant where Garst Road r[illegible] adjacent to the Sea).

Black-necked stilt nests monitored in this study were located in May and June of 1993. Nests were located along dike roads by sighting incubating adult black-necked stilts from a vehicle.

Nests were indirectly marked with a numbered piece of flagging attached to a flat metal washer placed 20 m south of nest bowls. Eggs were emersed in water to determine the sequence in which eggs were laid. For consistency, the first egg laid was collected for contaminant analysis and all remaining eggs were returned to the nest bowl. When possible, egg handling took place without the observer leaving the vehicle, to minimize the possibility of leaving a scent trail for predators. Nests were monitored at weekly intervals. When incubating [illegible] no longer present, nests were checked for hatching. Nest bowls with signs of predation or [illegible] sign of eggshell fragments were considered nests which had been preyed upon and eliminated from further hatchability analysis. Unhatched eggs were also collected for contaminant analysis.

<u>Study 3. Desert Pupfish Exposure Evaluation using Sailfin Mollies as Surrogates</u>

The desert pupfish was listed as a state endangered species by the CDFG in 1980, and as a federally endangered species by the U.S. Fish and Wildlife Service in 1986. The species current distribution is limited to [illegible] pools of the Salton Sea, two natural streams, eight artificial ponds, and [illegible] irrigation drains discharging into the Salton Sea. Because of the extremely limited range of this species, degradation of its habitat that could limit its reproductive capacity is an important issue. However, because the pupfish is a state and federal endangered species it could not be extensively sampled for this study. Therefore, a surrogate species was sampled instead of desert pupfish. Sailfin mollies (*Poecilia latipinna*) were collected to represent the contaminant burdens of the endangered desert pupfish. Of the fish species inhabiting irrigation drains, the sailfin molly was thought to be a reasonable surrogate for the pupfish. Sailfin mollies are native to the Southeastern U.S. and Mexico. Mollies are primarily vegetarians that feed on a variety of algae and detritus. Sailfin mollies have been introduced to the Salton Sea area where they are thought to compete with desert pupfish due to similarities in feed habits (McGinnis 1984).

Sailfin mollies sampled in this study were primarily collected during a [illegible]pfish survey conducted in 1994 by the Imperial Irrigation District (IID). Some additi[illegible]ollections were conducted by USFWS biologists using methods similar [illegible] those employed in th[illegible] survey (Remington and Hess 1993). Fish collections were [illegible]cted from July 20 to August 12, 1994. Sailfin mollies were collected from 13 drains (Figure 3). [illegible]sh minnow traps were set at locations along the last 2-5 km of a drain b[illegible]tered the [illegible] Sea. Traps were placed in slow-moving areas of water at d[illegible]ths of ab[illegible] to 1 meter. Traps were baited with cat food in perforated, se[illegible] plastic bags. The traps were left in place for approximately 4 to 24 hours, then retrieved and their contents recorded. Up to seven traps were placed alo[illegible] lower po[illegible] a drain f[illegible]es of the IID desert pupfish survey, but for this study [illegible]re colle[illegible] only three traps generally located at upstream, midpoint, and downstream [illegible] of the tr[illegible]. In most cases, approximately 20 to 60 grams of whole body m[illegible]lies (several [illegible] taken from a trap and composited into a chemically clean glass jar [illegible]d frozen until analysis. In one instance (at a trap on Trifolium Drain 13) only a single fish [illegible]ollected, so in th[illegible] case only a metal scan analysis was conducted on that sample. All other s[illegible] both a metal scan analysis and an organochlorine scan analysis. Chemical concentrations in the mollies from each of the three trap locations along a drain were averaged to determine the geometric mean chemical content of mollies in that drain. In one drain (W Drain) there was unexplained mortality of desert pupfish in two traps. That situation presented the opportunity to conduct contaminant analysis on desert pupfish to compare with mollies collected at the same location.

Analytical chemistry methods for the bird and fish tissues is detailed in [illegible]ix 1. Geometric means and ranges for these data are provided in Tables 3, [illegible]5 and 7 as [illegible] for chemical concentration data.

Results and Discussion

Study 1. Colonial Waterbird Eggshell Thickness[illegible]oxicity and [illegible] Contamination

Relatively low numbers of colonial waterbirds were [illegible] Salton Sea in 1993 and eggs for this portion of the study were collected opportunistically and included failed to hatch eggs (William Radke, SSNWR Biologist, personal communication). In some cases, two eggs were collected from a nest. A total of 35 great [illegible] were collected from 29 nests, 24 snowy egret eggs [illegible]cted from [illegible], and 17 black-crowned night-heron eggs were collected from 13 nests. [illegible]t egret [illegible] were collected from locations at the southern end of the Salton Sea (Poe and La[illegible] Roads), but all other egret and heron eggs were collected from the northern end of the Sea at the Whitewater River colonies. All the eggs were measured and the embryos removed and observed for gross deformities. The contents from 10 great egret and [illegible]ggs were submitted for further analysis including a detailed examination for malformed embryos and chemical analysis.

In calculating average eggshell thickness measurements for each species, averages were first calculated for sibling eggs from the same nest, and then the nest averages were combined for the species average. The average eggshell thickness measurements for the snowy egret was 0.207±0.024 mm (n=21), 0.282±0.024 mm (n=29) for great egrets, and 0.247±0.01[illegible] (n=13) for night-herons.

Little information is available regarding normal shell [illegible]ckness [illegible] these spec[illegible] environments, but Ohlendorf and Marois (1990) report m[illegible] thickness for night-heron eggs collected before 1947 (i.e., pre-DDT) was 0.280±0.01[illegible] at San [illegible] Bay and 0.266±0.15 at the San Joaquin Valley. In the present Salton Se[illegible]e night-he[illegible]gg shells were 7-12% thinner than the pre-DDT era night-heron [illegible]ggs from [illegible] Th[illegible] night-heron shell thinning observed in this study approaches th[illegible]ount ob[illegible]erved in [illegible]ke Ontario night-herons in 1972-1973 (14-17%) when hatching su[illegible]ess was [illegible]39% of [illegible]rmal (Price 1977). Davis (1993) presented a[illegible]mmary of p[illegible]ost- DD[illegible]gshell thickness for black-crowned night-herons fro[illegible] of stud[illegible]th America. The mean for the former was 0.284 ± 0.014. The mean thick[illegible]for blac[illegible]wned night-herons in this study was among the lowest of post- DD[illegible] era means a[illegible]er than all pre- DDT era means provided in that summary. In the[illegible]esent study, great e[illegible]ret eggs were 13% thicker than the mean shell thickness of 0[illegible]±0.016 mm rep[illegible]ed for 1985 Salton Sea great egret eggs that contained a geometric mean c[illegible]24 ppm WW DDE (Ohlendorf and Marois 1990).

Seventeen egret eggs contained embryos that could be examined for embryonic malformations. Of those, five embryos had some sign of deformity and three embryos exhibited some signs of embryo toxicity but did not show defects. The malformations observed in the egret embryos are detailed in Table 2. Although the observed beak defects could be in keeping with selenium terata, other chemical agents are also capable of causing them, and, in general, the embryos examined did not present the multiple deformities involving the eyes, [illegible] that are typical of classic selenium-induced teratogenicity (Dr. David Hoffman, NBS, P[illegible] Environmental Research Center, Laurel, MD and Dr. Joseph Skorupa, USFWS, Sacramento, CA, personal communication). A single egret embryo (Great Egret [illegible]) have multiple malformations and was a co-joined twin (two embryos joined at the [illegible] cavity with a single head. However, the twinning malformation is not typical of selenium terata (Dr. Joseph Skorupa, personal communication). The phenomenon of twinning (duplicity) has previously been described in common tern (*Sterna hirundo*) embryos and in that case was attributed to organochlorine contamination (Hays and Risebrough 1972). Therefore, organochlorine contamination (including, but not limited to, DDE) may be related to the unusual malformation observed in the single egret embryo.

The apparent high incidence of abnormal embryos in fish-eating birds nesting at the Salton Sea is a situation that merits additional investigation. When considered together, the rate of embryo [illegible] in this study in great and snowy egret eggs was 29 percent (5 of the 17 egret eggs containing embryos that were of adequate condition and stage of development for examination). If this incidence of abnormal embryos is actually representative of the Salton Sea population of fish-eating birds, then the rate is approximately 100 times higher than the typical incidence of abnormal embryos (Skorupa, USFWS, personal communication). It would be desirable to conduct additional field work on egrets nesting at the Salton Sea to determine if rates of embryonic malformations are truly that high, or were a function of the small nu[illegible]er of embryos examined in this study, and to compare these rates with egrets [illegible] at other wetland locations in the Imperial Valley. If the situation describe[illegible] this study [illegible]ssible management solution would be to encourage egrets to n[illegible] at alte[illegible]tive, less h[illegible] locations, if they exist.

Geometric mean concentrations of the principle [illegible]nts in the [illegible] and great egret eggs collected at the Salton Sea in 1993 are pre[illegible]nted in T[illegible] [illegible]erage moisture and lipid content in the snowy egret eggs was 80[illegible] and 5.5[illegible], respect[illegible]ely, and the average moisture and lipid content of the great egret eg[illegible] was 76.[illegible]d 6.2 %[illegible]espectively. The geometric mean selenium c[illegible]ntration w[illegible]ug/g DW [illegible].51 to 8.32) in the snowy egret eggs and 7.14 ug/g DW ([illegible]to 9.9) i[illegible] egret eggs, which places those populations at the Level of Concern (3-8 p[illegible]nium to[illegible] (BOR 1993). In 1985, 10 black-crowned night-herons and 10 [illegible]at egret eggs [illegible]cted from the Salton Sea area, and (when converted to DW) the[illegible]ontained geometri[illegible]ean concentrations of 4.4 (range 3.7-5.6) and 2.6 ug/g (2.2-3.3) s[illegible]um, respectivel[illegible] In this study great egret eggs on average contained over 2.7 times the a[illegible]m than eggs observed in 1985. On a individual egg basis, birds are at increased risk of reproductive failure when selenium concentrations in the egg is 5.1-20 ppm

(Skorupa et al. 1992). Therefore, 30% of the snowy egret eggs and 100% of the great egret eggs were in that reproductive depression range due to their selenium concentrations.

The geometric mean DDE concentration in snowy egret eggs was 6.33 ug/g WW ([illegible]ge 1.7 to 41) and the geometric mean in great egret eggs was 13.11 (range 4.5 to [illegible] (T[illegible]le 3). This mean concentration is below that found in 1985 by Ohlendorf a[illegible] Marois (19[illegible] [illegible]t egrets (24.[illegible] ug/g WW), but the maximum in this study was slightly [illegible]her tha[illegible] the maximu[illegible] measured in that study. The maximums from both snow[illegible]nd great egrets measured in this study exceeded the maximum of 20 ug/g WW mea[illegible]ured in [illegible]owned night-herons by Ohlendorf and Marois (1990). The mean for bl[illegible]ed night-[illegible] in that study was 8.62 ug/g WW, between the two means for the [illegible]ecies ex[illegible] [illegible]e geometric mean DDE contamination in Salton Sea snowy e[illegible]ts appro[illegible]hes and [illegible]t in great egrets exceeds the 8.0 ug/g level associated with reduce[illegible]eproduct[illegible] success [illegible] night-herons (Custer et al. 1983, and Henny et a[illegible]84). Near[illegible] the egre[illegible]ntained from 1.5 to 6 times the amount of DDE asso[illegible] reprodu[illegible]cts in night-herons (reproductive effects associated with DDE include eg[illegible]ning, egg[illegible]akage, and reduced clutch size, hatching success and subsequ[illegible]t productivity[illegible]tion, this study observed exceptionally high concentrations of DD[illegible] in some egret eggs[illegible] with one snowy egret egg containing 41 ug/g WW DDE and a great e[illegible]egg containing 5[illegible] ug/g. This was twice the amount of DDE observed in a great egret egg [illegible]able by Ohlendorf and Marois (1990) because it contained 24 ug/g WW DDE in 1985. Three other snowy egret egg samples and six other great egret egg samples individually exceeded the 8.0 ug/g level described by Custer et al. (1983) and Henny et al. (1984).

The geometric mean boron concentration in great egret eggs was 0.73 ug/g DW and the single snowy egret with measurable quantities of boron contained 0.77 ug/g DW (Table 3). Because these amounts are below the 3 ug/g DW level associated with reduced weight gain in ducklings (Smith and Anders 1989), egrets do not seem to be at risk to boron toxicity.

Toxaphene is a persistent organochlorine pesticide that w[illegible] present in m[illegible]gret eggs (Table 3). The geometric mean toxaphene concentratio[illegible]n great [illegible]rets eggs w[illegible] WW. This value was nearly 5 times higher than the geometric [illegible]centration of toxaphene (0.140 ug/g WW, range ND-0.33) observed in egret eggs fro[illegible] the Sa[illegible]n 1985 (Ohlendorf and Marois 1990). Toxaphene was banned for use i[illegible]EPA 198[illegible] has a half life in soil of up to 11 years (Eisler and Jacknow 198[illegible]. Toxap[illegible]own to cause severe embryonic effects including dislocat[illegible]oints an[illegible] poor gro[illegible]h in mallard ducklings when used at application rates in excess of 1.1[illegible]g/ha (Ho[illegible]an and [illegible]stin 1982). Dieldrin and PCBs are two other persis[illegible] contamin[illegible] were als[illegible] measurable, but non-hazardous, quantities in many o[illegible] eggs, an[illegible]ults are also presented in Table 3.

Study 2[illegible]lack-Necke[illegible]ryotoxicity, Nesting Proficiency, and Egg Contamination

In [illegible]2, 38 black-necke[illegible] stilt eggs were collected from the Salton Sea area for embryonic and [illegible]cal analysis, bu[illegible]esting was not followed that year. Twenty of those eggs contained [illegible]d be examined in detail for malformations and all of the 38 stilt eggs collected in 1992 were submitted for chemical analysis. In 1993, 40 stilt eggs were collected from several locations around the Salton Sea. Twenty-eight of the stilt eggs collected contained embryos that could be examined in detail and all 40 eggs collected in 1993 were submitted for chemical analysis. Each of these 1993 nests was observed for nest proficiency (defined as producing a full clutch of viable eggs) and those results are presented below.

The principal contaminants observed in each of the stilt eggs collected [illegible]92 and 1993 are presented in Tables 4 and 5, respectively. On a populatio[illegible]asis, the 19[illegible]s contained [illegible] geometric mean of 6.60 ug/g DW selenium (range 3.74[illegible]14.2) a[illegible] the 1993 st[illegible]ained a geometric mean of 5.82 ug/g DW selenium (range 3.67[illegible] These levels indicate the Salton Sea black-necked stilt populations are, on avera[illegible]e, at th[illegible]f Concern for bird hazard (BOR 1993).

The number of stilt eggs collected fr[illegible]nests in [illegible]92 and 1[illegible]93 considered at increased risk to hatching failure because of seleni[illegible] toxicity [illegible] presente[illegible] in Figure 3. In both years of the study, 95% of the[illegible] eggs sam[illegible]tained [illegible]elenium, meaning the nests those eggs came from were [illegible] more li[illegible]il than if the eggs contained less than 4.2 ppm selenium (Ohlendorf et al[illegible]rupa 19[illegible]

The[illegible]ilt eggs also conta[illegible]ed 0.47 ug/g DW boron in 1992 and 1.09 ug/g DW boron in 1993 ([illegible]s 4 and 5). Bo[illegible] of these geometric mean concentrations are below the 3 ug/g DW t[illegible] boron affects to duckling growth (Smith and Anders 1989).

The stilt eggs also contained geometric mean concentrations of 2.02 ug/g WW DDE in 1992 and 2.48 DDE in 1993. In 1993, when 40 stilt eggs were sampled, a single stilt egg was found to contain 23 ug/g WW DDE, a value twice as high as the 12.0 ug/g WW maximum observed when 84 stilt eggs were analyzed in the 1988-90 NIWQP irrigation drainwater study of the Salton Sea area (Setmire et al. 1993). Levels of DDE associated with reproductive [illegible] in black-necked stilts is not specifically known, but adverse effects occur in many fish-e[illegible] at <10 ppm DDE.

One stilt egg collected in 1992 (Egg ID 11) had signs of embry[illegible] that included hemorrhaging of the throat and had a selenium c[illegible] 3 ug/g D[illegible] [illegible]ble 4). One stilt egg collected in 1993 at Johnson Drain at the [illegible]th end o[illegible] Se[illegible] (Egg ID Johnson Drain 3) had a lower mandible slightly smalle[illegible] [illegible]lative to [illegible]e upper, [illegible]nd had a selenium content of 5.9 ug/g DW (Table 5). Neither of th[illegible]e malfor[illegible]tions by [illegible]emselves are considered selenium terata, as se[illegible]ium induce[illegible] [illegible]nities typ[illegible]lude multiple malformations of the eyes, bills and limbs.

The fate [illegible] the black-ne[illegible] nests monitored at the Salton Sea in 1993 is presented in Table 6. T[illegible]e black-necked sti[illegible] nest proficiency data set was analyzed to determine the proportion of [illegible] with reproducti[illegible] impairment by having 1 eggs that failed to hatch. Of the 37 nests [illegible] full term nests that were not preyed upon, abandoned or destroyed. However, four of those full term nests were excluded from further analysis because their fate was ambiguous in that at least one egg from each nest was known to have hatched, but the fate of the remaining eggs could not be determined (these four nests are indicated in Table 5 as having hatched 1+ eggs). One full term nest (Elmore Ranch 1) was observed to initially contain a full clutch of eggs and, although the fate of two eggs in that nest was unknown, the nest did contain one egg that failed to hatch. Two other nests each contained one failed-to-hatch eg[illegible] (Davis Road 10 and Hazard Tract 18). Therefore, three of the 23 full term nests had [illegible]to-hatch eggs, or 13% of the black-necked stilts nests studied were affected [illegible] hatching f[illegible]

Normally, only 8.9% of stilt nests with less than 4.1 ppm [illegible] in their eggs have ≥1 fail-to-hatch eggs, or, conversely, 91.1% of normal nests are u[illegible]affecte[illegible]to hatch eggs (USFWS 1995). When the Salton Sea black-necked stilt p[illegible]y (87.0% [illegible]cted nests) is compared to normal productivity, it was ca[illegible]ulated t[illegible] [illegible]pulation had 4.5% reproductive depression when compa[illegible] to stilts [illegible] seleniu[illegible]-normal environments [(91.1-87.0)/91.1 =0.045]. This amoun[illegible] reprodu[illegible]ve depre[illegible]on in the Salton Sea black-necked stilt population [illegible]ttributed to [illegible]nium co[illegible]ns in the eggs because 1) the reproductive success ca[illegible] were bas[illegible]n data from full-term nests that did not fail due to predation, distu[illegible]bandon[illegible]nd 2) selenium was the single contaminant measured at known [illegible]production ha[illegible]hold concentrations in the stilt eggs.

T[illegible]fore, there is a[illegible]pparent small (4.5%) effect in black-neck stilt reproduction that is c[illegible]own exposure-response data, and there is good reason to consider it biologically real. However, due to the small magnitude of effect, and the low statistical power of the sample size of 23 nests, the possibility that the result represents pure chance cannot be rejected. For example, a chi-square comparing the null expectation of 2.0 impaired nests (and 21.0 unimpaired, i.e., 8.9% impaired, the background rate) against the observed ratio of 3 impaired nests and 20 unimpaired nests yields a chi-square value of <1.0 (d.f.=1), which does not approach statistical significance at the 5% level. Based on the observed rate of im[illegible]rments observed, it has been calculated that a sample size of about 225 full ter[illegible] would need to be monitored in order to statistically verify a 4.5% reduction [illegible] nesting pro[illegible] Monitoring th[illegible] sample size of black-necked stilts is not realistic for any [illegible]ven yea[illegible]t the Salto[illegible]er, it could realistically be achieved over several nesting seaso[illegible]

The avian reproductive hazard in the Salton Sea [illegible]eviewed i[illegible]esentation at the 1994 Salton Sea Symposium (Skorupa 1994). B[illegible]cause th[illegible]relation between waterborne selenium and egg selenium in nonmi[illegible]ory spe[illegible]s of wat[illegible] birds, and a close correlation between egg selenium and toxic [illegible]cts, toxi[illegible]y thresh[illegible]s for avian eggs can be used to estimate toxicity thr[illegible]lds in wat[illegible]e diet. T[illegible]tionships and estimates have been independe[illegible]alidated [illegible]cent studies of avian exposure to selenium (USFWS 1995), therefore [illegible]l toxico[illegible]al relationships documented for the San Joaquin Valley also ap[illegible]r to apply to [illegible]the Salton Sea. For example, water-to-egg selenium ratios for bla[illegible]necked stilts docu[illegible]ented for the Salton Sea fall on the same regression line as data from [illegible]rson Reservoir [illegible]d the Tulare Basin (Dr. Joseph Skorupa, personal communication). Using [illegible]t could be predicted that approximately 11.9% (or, when rounded to the nearest whole number of nests for this sample set, three) of Salton Sea stilt nests would be affected by hatching failure. In fact, 13% (i.e., three) of Salton Sea stilt nests were observed to be affected by hatching failure in this study. Therefore, black-necked stilts nesting at Salton Sea appear to display the same selenium exposure response described for birds in other localities (Skorupa 1994, USFWS 1995). This demonstrates again the taxonomic and geographic robustness of the selenium toxicity thresholds currently established for birds, and that a large amount of predictive information about bird reproductive hazard can be obtained simply by knowing the concentration of selenium in the environment and the egg.

This study demonstrated that black-necked stilts are a good [illegible] to monitor for selenium-induced effects at the Salton Sea because: 1) no other contaminants [illegible] observed in their eggs at concentrations high enough to be a known reproductive impairment, [illegible]) the biological response of the stilts to the selenium exposure is apparently consistent with exposure-response data, although the sample size of Salton Sea stilt nest success data is small relative to desirable statistical power. Therefore, it would be desirable to continue monitoring black-necked stilts in the Salton Sea area to des[illegible] selenium e[illegible] the area s waterbirds, and more clear conclusion[illegible] reached w[illegible] breeding seasons.

<u>Study 3[illegible] Desert Pupfis[illegible] [illegible]sing Sailfin Molly Surrogates</u>

Sailfin mollies were trapped in 13 agricultural drains. The principal contaminants detected in s[illegible] mollies and desert pupfish collected in drains around the Salton Sea in 1994 are presented i[illegible]metric mean boron concentrations in the fish ranged from 3.3 to 28.0 ug/g DW, with the maximum concentration (32.3 ug/g) observed in mollies from Trifolium 12 Drain. It is not possible to compare these boron results with those of the NIWQP Detailed Study (Setmire et al. 1993) because in that study the boron reporting limit in sailfin molly tissues was 18 ug/g. The results are difficult to evaluate further because there are no boron criteria or effect levels for fish. Geometric mean DDE concentrations in mollies ranged from 0.03 to 0.93 ug/g WW, with the higher DDE concentrations seen in fish collected from the Vail 7 Drain (0.51 ug/g DDE), the R Drain (0.59 ug/g DDE), and the Trifolium 23 Drain (0.93 ug/g DDE). These concentrations of DDE are not hazardous to the fish themselves, but some approach the National Academy of Sciences 1 ug/g organochlorine pesticide threshold for protection of fish-eating [illegible] 1972). Also, the more elevated DDE concentrations probably indicate the relative DDE contamination in a particular drainage system. This information can be utilized to determine if particular drainages are contributing more bioavailable organochlorines to the ecosystem than others.

In the case of W Drain, where both sailfin mollies and desert pupfish were collected and submitted for analysis, there [illegible] generally [illegible] in contaminant levels between the two species, particularly in selenium. [illegible] mollies contained 5.6 ug/g DW and desert pupfish contained 5.4 ug/g [illegible]. Therefore, it seems that sailfin mollies are reasonably good indicators for desert pupfish selenium [illegible]ation loads.

Sailfin mollies from ten of the 13 drains and the desert pupfish from one of those ten drains [illegible] to 6 ppm DW selenium and so were at the Level of Concern for warmwater fishes (BOR 1993). Sailfin mollies in two other drains (Avenue 76 and Trifolium 18) contained 6.4 and 10.2 ug/g selenium, respectively, and so were over the toxicity threshold (>6 ppm) for warmwater fish reproductive hazards. Selenium hazards to desert pupfish is not precisely known, but they may have a sensitivity similar to that of the fathead minnow, another warmwater fish in the same family, Cyprinidae (Dr. Steve Hamilton, National Biological Service, personal communication). Juvenile fathead minnows exhibit growth inhibition at whole bo[illegible] concentrations of 6 to 8 ug/g DW selenium (Bennett et al. 1986, and O[illegible] Knight 1989), and a significant increase in edema and lordosis (curved spine[illegible] larval fat[illegible]ws when adults where exposed to selenium water at concentratio[illegible]s low [illegible]10 ug/L. L[illegible] concluded that 4 ug/g DW selenium be considered the to[illegible] threshold for the overall health of and reproductive vigor of freshwater fish. Therefor[illegible] the de[illegible]sh is apparently at reproductive risk in many of the drains where th[illegible]wn to occu[illegible]

Finally, fish collected from all drains [illegible]ceeded e[illegible]her the L[illegible]el of Concern (2-6 ppm) or Toxicity Threshold (>6 ppm) for dietary c[illegible]ria, indi[illegible]ng that [illegible]sides the risk to the fish themselves, the fish also pr[illegible]t a risk to [illegible]s that w[illegible]sume them.

Conclusions a[illegible]endati[illegible]

The am[illegible]t of eggshel[illegible](up to 12%) observed in black-crowned night herons nesting at the [illegible]lton Sea in 1993 i[illegible]icates that species is likely to be experiencing reproductive depression [illegible]d to egg failures[illegible] Embryonic malformations were observed in 29% of the snowy egret and g[illegible]os examined in detail. A variety of defects were observed, including the unusual malformation of a twin embryo joined at the body but with a single head, but none of these malformations were considered typical selenium-induced terata. The deformities observed could be related to the multiple kinds of contaminants observed in the egret eggs, but these kinds of synergistic effects are poorly understood. Chemical analysis of the egret embryos indicated the egg selenium content ranged from 3.5-9.9 ug/g DW, levels that put the birds at risk to lowered productivity but unlikely to produce observable rates of teratogenicity in th[illegible] small number of egret eggs examined. Therefore, neither the kinds of deform[illegible] [illegible]erved in the egrets, nor the associated levels of selenium in their eggs [illegible]icate that s[illegible]duced terata i[illegible] occurring in egrets nesting at the Salton Sea area. How[illegible]r, the e[illegible]t eggs also [illegible]gh levels of DDE with a geometric mean of 6.33 ug/g in th[illegible]grets and 13.11 ug/g in the great egrets. These levels of DDE approach and excee[illegible] the am[illegible]g/g) associated with reduced reproductive success in black-crowned [illegible]s (Custer [illegible] 1983, and Henny et al. 1984). An additional concern is that some [illegible]grets co[illegible]gly higher levels of DDE and toxaphene in this study than in an[illegible]er stud[illegible]f contam[illegible]nants in egrets from the Salton Sea in 1985. Apparently, high levels [illegible] these pe[illegible]stent co[illegible]minants are still available to some of these birds[illegible]tmire et al[illegible] identifie[illegible]ontamination at all trophic levels (including resident sp[illegible]he Salto[illegible]ystem. In fact, it was detected in 99% the samples analyzed, and co[illegible]ns in bio[illegible]re correlated with trophic level. That study concluded that resi[illegible]nt species of [illegible] Imperial Valley are likely to experience reproductive im[illegible]rment as a result o[illegible]he DDE contamination. Other sources of DDE are potentially a[illegible]ble to migrator[illegible]species, including possible sources in Mexico. However, a recent study by [illegible]ates that there is no clear evidence for increased bioaccumulation of DDE for migratory species while wintering in Mexico. The limited data suggest that bioaccumulation is similar in Mexico and the Southwestern United States. The reported declines in colonial nesting bird success at the Salton Sea is likely to be related to the high levels of multiple contaminants in these fish-eating birds, particularly organochlorines.

The status and nesting success of colonial waterbirds in the Salton Sea area needs additional and routine evaluation. The extent to which contaminants-induced reproductive failure is influencing nesting success of fish-eating birds needs to be determined and separated from other demographic factors such as nest-site fidelity and adequacy of food supply. A[illegible]ations in the Salton Sea area of these species should be monitored and eggs from discrete colonies should be randomly sampled to more precisely determine rates [illegible] of malformations, and be analyzed for associated contamination. Fledging success from each colony should be calculated to determine population trends for these species in the Salton Sea area, and to determine if birds utilizing particular nesting areas are at greater risk to contaminant exposure that could cause reproductive failure. Although more site specific information is desirable regarding contaminant effects on fish-eating birds in the Salton Sea area, it is highly desirable to reduce the [illegible]bility of [illegible] the Imperial Valley and Salton Sea ecosystem. However, because of the multiple contaminant exposures of the colonial waterbirds, they are not the ideal species to monitor for s[illegible]elated hazard information in the Salton Sea area.

The black-necked stilt study indicates that this species is likely to be experiencing selenium-induced reproductive depression. Nesting proficiency of Salton Sea area stilts was 4.5% lower than that reported for stilts with low selenium exposure (USFWS 1995), with 13% of the full term stilt nests affected by having at least one egg that failed to hatch. The geometric mean selenium content of the stilt eggs (6.60 ug/g in 1992 and 5.82 ug/g in 1993) places the stilts at a four-times greater risk to reproductive depression due to egg mortality than if they had selenium levels below 4.1 ppm (Skorupa 1994). The stilts at Salton Sea did not exhibit selenium-induced terata, but the likelihood of observing embryo deformation at those eggs selenium concentrations and the samples sizes of this study would be very low (Dr. Joseph Skorupa, personal communication). Therefore, when assessing the hazard of selenium to [illegible] Salton Sea, the most appropriate type of investigation is a nesting proficiency study with chemical analysis of one egg from each nest.

One aspect that needs to be considered when evaluating the data for black-necked stilts is their level of sensitivity to selenium toxicity. Stilts are moderately sensitive to the reproductive effects of selenium toxicity as compared to ducks which are considered sensitive to these effects (Skorupa et al. 1996). While the effect measured here was small, an effect was detectable in a moderately sensitive species. This raises concerns about the potential for reproductive impairment [illegible] sensitive [illegible] that nest in the Salton Sea ecosystem.

The sailfin molly study [illegible] that the endangered desert pupfish is probably also at risk to reproductive hazards from selenium. In addition, the sailfin mollie data indicate that fishes in the agricultural drains are presenting a selenium hazard as a dietary food item.

To summarize the management implications of this study, the reproductive depression in birds due to both selenium and DDE, hazards to the endangered pupfish, and levels of selenium in fish as a dietary food item have emerged as the most serious concerns for fish and wildlife resources in the Salton Sea area. The biological hazards relative to persistent contaminants in the Salton Sea area are now more clearly understood and the information indicates which species and endpoints are most relevant to efforts at improving and monitoring the Salton Sea contaminant issues, with respect to NIWQP responsibilities.

Remediation efforts need to focus on reducing levels of selenium and DDE con[illegible] biota to below hazardous effects levels. For example, a management goal of reducing in drainwater to <10 ppb water-borne selenium has been recommended to prevent most avian toxicity, although not avian contamination (Skor[illegible] Ohlendorf [illegible]). It would be necessary to determine if that avian management goal would also [illegible] adequate protection for desert pupfish. This management goal could be aligned with proposed investigations in the Imperial Valley to identify drainages with the highest [illegible]t and selenium loads and recommend effective technologies to limit or re[illegible] contaminants from those drainage systems (Setmire, USGS, personal communication). The information obtained in this study provides guidance for focused assessment measures to determine if remediation efforts are effective in limiting or reversing biological hazards from [illegible] exposure. Monitoring selenium concentrations in black-necked stilt eggs and sailfin mollies whole bodies would be appropriate biological endpoints to evaluate the success of selenium remediation efforts in the Imperial Valley. Monitoring [illegible] concentrations in a fish-eating species of bird, such as a heron or egret would be appropriate to evaluate the success of efforts to limit DDE bioavailability in the aquatic system.

References

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis, 15th Ed., Methods 926.08 and 925.09. Arlington, Virginia.

Audet, D.J., M. Shaughnessy, and W. Radke. 1997. Org[illegible]chlorine a[illegible] in fishes an[illegible] colonial birds from the Salton Sea. Report submitted t[illegible]e U.S. [illegible]sh and Wil[illegible] Regional Office, Portland, Oregon. 18 pp.

Bennett, W.N., A.S. Brooks and M.E. Boraas. 1[illegible]nium Upt[illegible]d Transfer in an Aquatic Food Chain and Its Effects on Fat[illegible]ad Minn[illegible]w[illegible] *[illegible]. Environ. Contam. Toxicol.* 15:513-517.

Bureau of [illegible]amation. 1[illegible]emorand[illegible]tional Irrigation Water Quality Program Manager re[illegible]redicted [illegible] Effect Levels for Kendrick and Middle Green River Remediation (Ir[illegible]ainage).

Co[illegible], J.J., D.R.Buckler, C.G. Ingersoll, J.F. Fairchild, and T.W. May 1993 Effect of Dietary S[illegible]ium on the Rep[illegible]ductive Success of Bluegill Sunfish *(Lepomis macrohirus). Environ.* [illegible]: 551-565.

Cumbie, P.M. and S.L. Van Horn. 1978. Selenium Accumulation Associated with Fish Mortality and Reproductive Failure. *Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies* 32: 612-624.

Custer, T. W., G.L. Hensler, and T.E. Kaiser. 1983. Clutch size, reproductive success, and organochlorine contaminants in Atlantic Coast black-crowned night-herons. *Auk* 100:699-710.

Dahlquist, R.L. and J.W. Knoll. 1978. Inductively-coupled plasma-atomic emission spectrometry: Analysis of biological materials and soils for major, trace, and ultra-trace elements. *Applied Spectroscopy*, 32(1):1-29.

Davis, W.E., Jr. 1993. Black-crowned Night-Heron (*Nycticorax nycticorax*). *In* The Birds of North America, No. 74 (A. Poole and F. Gill (eds.). Philadelphia: The Academy of Natural Sciences; Washington, D.C.: The American Ornithologists' Union.

Eisler, R. And J. [illegible]. 1985. Toxaphene Hazards to Fish, Wildlife and Invertebrates: a Synoptic Review. U.S. Fish and Wildlife Service Biological Report 85(1.4), Washington, D.C.

Gillespie, R.B. and P.C. Baumann. 1986. Decline of Bay-Delta Fisheries and Increased Selenium [illegible] Reproduction by Bluegills. *Trans. Amer. Fish. Soc.* 115:208-213.

Gruenwald, P. 1958. Malformations caused by necrosis in the embryo illustrated by the effect of selenium compounds on chick embryos. *Amer. J. Pathol.* 34:77-103.

Hamilton, S.J., A.N. Palmisan, G.W. Wedgemeyer and W.T. Yasutake. 1986. Im[illegible]cts of Selenium on Early Life Stages and Smoltification of Fall Chinook Salm[illegible] *T[illegible]ans. North Amer. Wildl. and Nat. Resour. Conf.* 51:343-356.

Hamilton. S.J., K.J. Buhl and N.L. Faerber. 1989. Toxic[illegible]lenium in the diet to chinook salmon. In A.Q. Howard (ed.). *Selenium and Agricult[illegible]al Dr[illegible]plications for San Francisco Bay and California Environment, Pro[illegible]f the Four[illegible]enium Symposium. The Bay Institute of San Francisco*. Tibur[illegible], CA pp 22[illegible]

Hamilton, S.J., K.J. Buhl, N.L. F[illegible]ber, R.H. [illegible]iedmey[illegible], and F.A. Bullard. 1990. Toxicity of Organic Se[illegible]um in the D[illegible]hinook S[illegible] *[illegible]nviron. Toxicol. Chem.* 9:347-358.

Hays, H. and R.[illegible]ugh. 19[illegible] Pollutant concentrations in abnormal young terns from Long Is[illegible]d Sound. *Au[illegible]*[illegible]5.

[illegible]y, C.J., L.J. Blu[illegible] A.J. Krynitsky, and C.M. Bunck. 1984. Current impacts of DDE [illegible] night-herons in the intermountain west. *J. Wildl. Manag.* 48:1-13.

Hermanutz, R.O., K.N. Allen, T.H. Roush, and S.F. Hedtke. 1992. Effects of elevated selenium concentrations on bluegills (*Lepomis macrochirus*) in outdoor experimental streams. *Environ. Toxicol. Chem.* 11:217-224.

Hilton, J.N., P.V Hodson, and S.J. Slinger. 1980. The requirement an[illegible] of selenium to rainbow trout (*Salmo gairdneri*). *J. Nutrition* 110:2527-[illegible]5.

Hodson, P.V. and J.W. Hilton. 1983. The nutritional req[illegible]s and toxicity to fish of dietary and waterborne selenium. *Environ. Biogeochem. Ecol.* [illegible]*ull.* (S[illegible]) 35:335-340.

Hodson, P.V., D.J. Spry, and B.R. Blunt. [illegible]80. Eff[illegible]rout (*Salmo gairdneri*) of a chronic exposure to waterborne sele[illegible]n. *Can.* [illegible] *Fish Aq*[illegible]*t. Sci.* 37:233-240.

Hoffman, D[illegible]and W.C. E[illegible]r. 1982. [illegible] lindane, paraquat, toxaphene, and 2,4,5-trichloroph[illegible]tic acid o[illegible]d embryo development. *Arch. Environ. Contam. Toxicol.* 11:79-86.

Hu[illegible], J.B., S.J. Hamilto[illegible] and D.R. Buckler. 1987. Toxicity of sodium selenite to rainbow trout f[illegible]*ater Res.* 21(2[illegible]33-238.

Lemly, A. D. 1993. Guidelines for evaluating selenium data from aquatic monitoring and assessment studies. *Environ. Monit. Assess.* 28:83-100.

McGinnis, S.M. 1984. *Freshwater Fishes of California.* Univ. Of California Press. Berkeley. 316 pp.

Mora, M.A. 1997. Transboundary pollution: persistent organochlorine pesticides i[illegible]migrant birds of the southwestern United States and Mexico. *Environ. Toxicol*[illegible] 16(1):3-11.

NAS. 1972. National Academy of Sciences, National A[illegible]demy o[illegible] Engineering[illegible]). Water quality criteria, 1972. U.S. Environmental Protect[illegible]cy Report, EPA R3-73-033. 594 pp.

Ogle, R.S. and A.W. Knight. 1989. Effect[illegible] of elevat[illegible]enium on growth and reproduction of the fathead minnow (*P*[illegible]*ephale*[illegible]*promelas*[illegible] *Arch. Environ. Contam. Toxicol.* 18.795-803.

Ohlendorf, [illegible]989. Bio[illegible]tion and effects of selenium in wildlife. In: Selenium in Agriculture and [illegible]nment. [illegible] Science Society of America and American Society of Agrono[illegible] Special Pub[illegible] 23.

O[illegible]dorf, H. M. , an[illegible] K. C. Marois. 1990. Organochlorines and selenium in California night-[illegible]ggs. *Environ. Monit. Assess.* 15:91-104.

Ohlendorf, H.M., J.P. Skorupa, M.K. Saiki, and D.A. Barnum. 1993. Food-chain transfer of trace elements to wildlife. Pp.596-603 in R.G. Allen and C.M.U. Neale (eds.), *Management of Irrigation and Drainage Systems: Integrated Perspectives.* American Society of Civil Engineers, New York, NY.

Price, I.M. 1977. Environmental contaminants in relatio[illegible] Canadian [illegible] *Trans. North Am. Wildl. And Nat. Resour. Conf.* 42:382-396.

Pyron, M. and T.L. Beitinger. 1989. Effect of seleniu[illegible] on rep[illegible] behavior and fry of fathead minnows. *Arch. Environ. Contam. Toxi[illegible]*[illegible]9-613.

Remington, M. and P. Hess. 1993. Res[u]lts of D[e]sert Pupf[illegible]h Trapping in Imperial Irrigation District Drains. Imperial Irrigati[illegible] District, [illegible]lifornia.

Roberts, C.[illegible] Trace [illegible]d Organochlorine Contamination in Prey and Habitat of the Yuma Clapp[illegible]he Impe[illegible]alley, California. Report submitted to the U.S. Fish and Wildlif[illegible]ervice Regio[illegible] Portland, Oregon. 20 pp.

S[illegible] M. K. 1990. [illegible]mental concentrations in fishes from the Salton Sea, Southeastern [illegible], *Air, and Soil Pollut.* 52:41-56.

Schroeder, R.A., and M. Rivera. 1993. Physical, chemical, and biological data for detailed study of irrigation drainage in the Salton Sea area, California, 1988-1990. U. S. Geological Survey. Open File Report 93-83. 179 p.

Schultz, R., and R. Hermanutz. 1990. Transfer of Toxic Concentratio[illegible] S[illegible]enium from Parent to Progeny in the Fathead Minnow (*Pimephales pr[illegible]elas*). *Arc[illegible] Contamin. Toxicol.* 45:568-573.

Setmire, J.G., J.C. Wolfe, and R. K. Stroud. 1990. R[illegible]onnais[illegible]estigation of water quality, bottom sediment, and biota associated w[illegible]ion draina[illegible]he Salton Sea area, California, 1986-87. U.S. Geological Sur[illegible]y. Wate[illegible] Inv[illegible]stigation Report 89-4102. 68 p.

Setmire, J.[illegible]R.A. Schro[illegible]. Densm[illegible]Goodbred, D.J. Audet, and W.R. Radke. 1993. Deta[illegible] of wate[illegible]bottom sediment, and biota associated with irrigation drainage in the S[illegible]area, Ca[illegible]ia. U.S. Geological Survey. Water Resources Investig[illegible]ions Report 9[illegible]02 p.

S[illegible]pa, J.P. 1994. [illegible]pacts of selenium on the biological systems of the Salton Sea. In: *[illegible] Salton Sea Symposium,* January 13, 1994, Indian Wells, CA. Salton Sea Authority, Imperial, CA. 15 pp.

Skorupa, J.P. and H.M. Ohlendorf. 1991. Contaminants in drainage water and avian risk thresholds. In. Ariel Dinar and David Zilberman (eds.), *The Economics and Management of Water and Drainage in Agriculture.* Kluwer Academic Publishers.

Skorupa, J.P., H.M. Ohlendorf, and R.L. Hothem. 1992. Interpretive [illegible] for field studies of selenium-exposed waterbirds. Unsubmitted manuscri[illegible] 35 pp. + ta[illegible]ures.

Skorupa, J. P., S.P. Morman, J.S. Sefchick-Edwards. 19[illegible]elines for interpreting selenium exposures of biota associated with nonmarine aquatic [illegible]bitats. [illegible] for the National Irrigation Water Quality Program. 74 pp.

Smith, G.J. and V.P. Anders. 1989. T[illegible]ic effe[illegible] of boro[illegible]n mallard reproduction. *Environ. Toxicol. Chem.* 8:943-950.

U.S. Envir[illegible] Protectio[illegible]. 1982. Toxaphene, intent to cancel or restrict registrations of [illegible]ontaini[illegible]aphene; denial of applications for registration of pesticid[illegible] products conta[illegible]phene; determination containing the rebuttable presumption aga[illegible]t registration; avai[illegible]bility of decision document. *Fed. Reg.* 47(229):53784-53793.

U[illegible]al Protection Agency (USEPA). 1984. Test Methods for Evaluating Solid Waste, EPA Publication No. SW-846, 2nd Ed. U.S. EPA: Washington, D.C.

U.S. Environmental Protection Agency (USEPA). 1986. Test Methods for Evaluating Solid Waste-Physical/Chemical Methods, EPA Publication No.SW-846. Office of Solid Waste and Emergency Response: Washington, D.C.

U.S. Environmental Protection Agency (USEPA). 1987a. Ambient water quality criteria for selenium -1987. U.S. Environmental Protection Agency 440/5-87-006.

U.S. Environmental Protection Agency (USEPA). 1987b. Test Methods for Evaluating Solid Waste, EPA Publication No. SW-846, 3rd Ed. U.S. EPA: Washington, D.C.

U.S. Environmental Protection Agency (USEPA). 1990. Contract Laboratory Program, Statement of Work for organic analysis, multi-media, multi-concentration. Document Numbers OLM01.0 (March 1990), OLM01.1 (December 1990), and OLM01.2 (January 1991).

U. S. Fish and Wildlife Service. 1991. Colonial nesting bird survey - 1991 and summary since 1987. Salton Sea National Wildlife Refuge Memorandum, August 25, 1991.

U. S. Fish and Wildlife Service. 1992. Colonial nesting bird survey - 1992 and summary since 1987. Salton Sea National Wildlife Refuge Memorandum, August 17, 1992.

U.S. Fish and Wildlife Service. 1994. Aspects of the reproductive success of black skimmers and the relationship of DDE and selenium concentrations at the Salton Sea, 1993. 10 p.

U.S. Fish and Wildlife Service. 1995. Compensation habitat protocol for drainwater evaporation basins. January, 1995. Division of Environmental Contaminants, Sacramento Field Office, U.S. Fish and Wildlife Service, Sacramento, CA. 26 pp.

White, J.R., P.S. Hofmann, D. Hammond, and S. Baumgartner. 1987. [illegible]n verification study 1986. Final Report to the California State Water Re[illegible]urces Cont[illegible] Bay-Delta Project and Water Pollution Control Laboratory, Califo[illegible]a Depar[illegible]ent of Fish [illegible] Sacramento, California.

Wilber, C. G. 1980. Toxicology of selenium: a [illegible] *[illegible]linical Tox[illegible]* [illegible]7:171-230.

Woock, S.E., W.R. Garrett, W.E. Par[illegible] and W.[illegible] Bryson. [illegible]987. Decreased survival and teratogenesis during laboratory s[illegible]nium ex[illegible]ures to b[illegible]egill, *Lepomis macrochirus. Bull. Environ. C[illegible]m. Toxicol.* [illegible]8-1005.

Appendix 1

Analytical Chemistry Methods for Bird Tissues

ChemicalChemical analyses for inorganic compounds in birdChemical analyses f[illegible]inorganic compounds in bi InstitInstituteInstitute (ReInstitute (Research Triangle Park, NC). El[illegible]nalyzed included arsenic, alumin barium,barium, beryllium, cadmium, chromium, cop[illegible], iron, ma[illegible]manganese, [illegible]mercury, molybdenum,molybdenum, nickel, lead, selenium, st[illegible]tium, v[illegible]adium, an[illegible]sue samples were prehomogenizedprehomogenized using a food processo[illegible]rehomogenized using a food processor and determinationdetermination of moisture content anddet[illegible]inatio[illegible]isture content and ground through a 100-m microwavemicrowave oven,microwave oven, 0.5[illegible]freeze-drie[illegible]nples were heated inmicrowave oven, 0 ofof 5 ml of Baker Instra-Analyzed nitric ac[illegible] for thre[illegible]12[illegible]watts, then three minutesof 5 ml of Baker Ins watts,watts, and finally for 35 min[illegible] at 450 [illegible]atts. Ve[illegible]sel caps were rinsed intowatts, and finally for 35 additionaladditional nitric acid a[illegible] the unca[illegible]ed vessel [illegible]as radditional nitric acid and the uncapped vessel was volumevol[illegible] remaining[illegible] than 1 m[illegible] remaining was less than 1 ml. Samples werevolume remainin andand ce[illegible]and ce[illegible] precipitate the suspended material. Samples then underwent inductivelyan plasmaplasma s[illegible] (ICP) [illegible]sma spectroscopy (ICP) analplasma spectroscopy (ICP) analysis (USEI LabsLab[illegible] Plasma Spec[illegible]tial Labs Plasma Spec I sequential or Labs Plasma Spec I sequential or ES20 gra[illegible]tegraphite furnace [illegible]r cold vapor atomic absorption were homogenigraphite furnace or cold vapor atomic a[illegible]sis.analysis. Th[illegible]eanalysis. These samples were similarly heated in a microwave, however the durationanaly [illegible]450 watts reduced to 15 minutes. heating at 450 watts reduced to 15 minutes. Residuesheat laboratorylaboratory pure water.laboratory pure water. Graphite furnace atomic absorption measurements for arse (USEPA(USEPA 1984) were (USEPA 1984) were made(USEPA 1984) were made with a Perkin-Elmer Z specspectspectrometer.spectrometer. Cold vapor atomic absorption measurements for mercury (USEPA 198

conducted using SnC_{14} as a reducing agent with a Leeman PS200 Hg Analyzer.

ChemicalChemical analyses for organic compounds in bird tissues were perfor[illegible]d at Mississippi State ChemicalChemical Laboratory (Starkville, MS). Organic analytes [illegible] included organochlorine pesticidespesticides (OCs), polychlorinatedpesticides (OC[illegible] polychlorin[illegible]yls (PCBs), [illegible]s-nonachlor,pest werewere thoroughly mixed with anhydrous sodium su[illegible]ewere t[illegible]oroughly mi[illegible]ydrous sodium sulfa hexanehexane for seven hours. Extracts were concen[illegible] rotary evaporation and concentrated todrynesstodryness for lipid determination. After weig[illegible]ng,tod[illegible] lipid determination. After weighing, sa extractedextracted four timeextracted four timese[illegible]ur times ex[illegible]d four times usingextracted four times us petroleum ether which was then washed, c[illegible]centrate[illegible],p[illegible]r which was then washed, concentrated, and columncolumn containing 20 g of Flo[illegible]l. The c[illegible]umn was [illegible]luted with 200-ml 6% diethyl ether and 94% petroleumpetroleum ether follo[illegible] by 200[illegible]troleum [illegible]her followed by 200-ml 15%petroleum ether followe fractionfra[illegible] was conce[illegible]o the app[illegible]olume for quantifraction was concentrated to the appropriate vo electronele[illegible]ture gase[illegible]pture gas chromatography using a Varian 6000/6500 or Varian 3600 gaselec TheThe first fra[illegible]he first [illegible]n was concentratedThe first fraction was concentrated and transferred to addition[illegible]additional cle[illegible]uired to separate PCB s from the other OC s. Three fraadditional clean-up req fro[illegible]om the silicic acid [illegible]lumn, and each was concentrated to the appropriate volume forfrom the silicic acid colu b[illegible]acked or mega[illegible]re columnby packed or megabore column electron capture gas chromatographyby packed [illegible]hromatograph.

Analytical Chemistry Methods for Fish Tissues

Chemical analyses for inorganic elements in fishChemical analyses for inorganic elements in fish tissues were pe America,America, Inc. (Madison, WI). Analysis includedAmerica, Inc. (Madison, WI). Analysis included quant tissuetissue analysis described above. Percent moisture wastissue analysis described above. Percent moisture was aluminumaluminum dish then drying in an ovenaluminum dish then dry oven at 100aluminum dish then d (AOAC(AOAC 1990). Elemental analysis was conducted ICP. Dige AC 1990). El mental analysis inin a microwave digester. Emission intensities were co paredin microwave mission intensities we usingusing a Thermo Jarrell Ash ICAP 61Eusing a Therm Ash ICAP 61E spectrometer, with the spectrom backgroundbackground and interbackground and inter ring e nd and interfering elements. Mercury an absorptionabsorption spectroscopy (USEPA 198 s were dig usingabsorption spectroscopy (USEPA thethe mercury was reduced with sodium b ohydride reduced with sodium borohydride for determ determineddetermined at waveleng of 253. nm usi g a Leemadetermined at wavelength of 253.7 spectrophotometerspectrophoton r withsp rophotor ter with an MHS-20 hydride generation unit, with the s solutions.s ons. Arsen elenium a weresolutions. Arsenic and selenium analyses were conducted spectroscop copy (U 4) on a Perkin-Elmer Zeemspectroscopy (USEPA 1984) on a Perkin-Elmer Z digesteddigested digeste nitric acid in a microwave digester. Arsenic was determined atdigested w andand elenium was selenium was determined at 19and selenium was determined at 196.0 nm em yedemployed in th mployed in the analysis, and standard additions wereemployed in the analysis, and standa

OrganicOrganic analyses of the fish Organic analyses of the fish samples Organic analyses of the fish samples w TheseThese methods included the determination of OCsThese methods included the determination of OCs andThe

deltadelta BHC. Percent delta BHC. Percent moisture was dedelta BHC. Percent moisture was determined b
aluminumaluminum pan. Samples were dried in an oven at 105 C for 16 hours and allaluminum pan. Samp
desiccatordesiccator before bedesiccator before beingdesiccator before being desiccator before being weidesiccator b
thethe percent moisture: [mass the percent moisture: [mass (g) pan +the percent [illegible]isture: [mass (g) pan + wet
samplesample = % moisture grams of sample. Spiking solutions are ad[illegible]e = % moisture grams of sample.
werewere ground. Pesticide spikes were added to a powe[illegible]ground. Pe[illegible]ikes were ad[illegible]d to a portwere
ofof of 2,4,5,6-tetrachloro-m-xylene were used as a bla[illegible] spike s[illegible]ution forof [illegible]chloro-m-xylene wer
samples were then dried under a hood using anhydrous s[illegible]lfate. A soxhlet extractor was
usedused with methylene chloride to extract the desired [illegible]action[illegible] samples (USEPAused with methylene c
resulting extracts were then concentrated in a K[illegible]nish appar[illegible] aresulting extracts were then concent
hothot water bath, then diluted tohot wate[illegible]ath, the[illegible]10 [illegible]l with methylene chloride. One ml subsam
werewere used for lipid determwere [illegible]d for li[illegible]d determ[illegible]ation.were used for lipid determination. Subsample
placedplaced under a hood to e[illegible]orate op[illegible]ed unde[illegible] hood to evaporate off the solvplaced under a hood t
equation u[illegible]toequation [illegible]alculate [illegible]pid: [(weight (g)equation used to calculate percent lipid: [(w
100/grams[illegible] of samp[illegible]pid grams extra100/grams of sample = % lipid grams extracted. Five 10
injectedinjected [illegible]C Labora[illegible] Modelinjected on an ABC Laboratories Model 1002B Gel-Permeation C
upup (U[illegible]EPA1990) us[illegible]n packed with 70 g of S-X3 Bio-beadsup (USEPA1990) using a column packe
the[illegible] carrier solvent. T[illegible]s extractthe carrier solvent. This extract was again concentrated to 5 ml. Then 50the c
t[illegible]e samples and t[illegible]ey were concentrated forto the samples and they were concentrated for a third time to a 5t
a[illegible] of PCBs from the OCs was carried out in a silica gel column. and separation of PCBs from the
elutedeluted witheluted with petroleum ether and the second fraction was extracted using a mixture of 1%eluted wit
19%19% hexane and 80% methylene chloride.19% hexane and 80% methylene chloride. 19% hexane and 80% meth

DanishDanish apparatus, followed by dilution with hexane and a repeat of the concentration step. The fractionsfractions underwent electron captufractions underwent electron capturefractions underwent electro chromatograph for quantification of individual constituents.

AnyAny use of trade, product, or firmAny use of trade, product, or firm [illegible] this report is for descriptive purp imply endorsement by the U.S. Government.