

**CONTAMINANTS IN PELICANS COLLECTED DURING THE  
AVIAN BOTULISM EVENT AT THE SALTON SEA IN 1996**

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## Introduction

The Salton Sea is located in the desert region of southern California in Riverside and Imperial Counties (Fig. 1). The current Salton Sea was created by an accidental misdirection of the Colorado River in 1905 into the Salton Trough. Since the river flow was contained in 1907, the Salton Sea has received the majority of its input from three rivers: the Alamo and New Rivers in the south and the Whitewater River at the north end. These rivers carry largely irrigation drainwater flows, but the New River also carries municipal and industrial sewage flows from Mexicali, Mexico. As a result of evaporative water loss (the only way water exits the Salton Sea), the Salton Sea has a salinity of 44 parts per thousand (ppt; Ogden Environmental and Energy Services, Inc. 1996) which is higher than that for seawater (35 ppt).

The Salton Sea has been the site of several fish and bird die-offs in recent years. In 1996 a major outbreak of Type C avian botulism occurred. The total confirmed bird deaths was over 14,000. American white pelicans (*Pelecanus erythrorhynchos*) comprised the majority of that at over 8,500 deaths. The brown pelican (*Pelecanus occidentalis*), a federally-listed endangered species, also succumbed to avian botulism in large numbers (over 1,100 deaths), although over 800 were saved through rehabilitation efforts (Ken Sturm, Salton Sea National Wildlife Refuge, pers. comm.). Avian botulism is not uncommon in the western United States (Locke and Friend 1987), but it was not previously known from fish-eating birds. The isolation of botulism toxin from live fish (which were also infected with a *Vibrio* bacterium which apparently created anaerobic conditions in the gut) had not occurred in the past and accounted for the unusual suite of species (particularly pelicans) that died in the event (Tonie Rocke, National Wildlife Health Center, pers. comm.).

While the cause of the die-off was avian botulism, there is potential for bird exposure to contaminants such as pesticides and selenium in the Salton Sea ecosystem. The purpose of this study was to evaluate if exposure to contaminants may have contributed to the die-off in brown and white pelicans. Other studies have demonstrated that wildlife have been exposed to contaminants in the Salton Sea ecosystem (Setmire *et al.* 1990 and Setmire *et al.* 1993), and some birds, such as black-necked stilts (*Himantopus mexicanus*) and great egrets (*Casmerodius albus*), may be subject to reproductive impacts as well (Bennett 1997). Reproductive impacts are not the main concern here, but rather the concentrations measured are considered relative to established toxicity thresholds in birds.

There is potential for immune suppression effects to have been a factor, but immune suppression by contaminants has only been studied for a small number of contaminants in birds (Ann Fairbrother, pers. comm.). Chronic contaminant exposure can result in increased metabolic rates thus requiring more energy for maintenance and leaving less available for disease suppression (Peterle 1991). In addition, heat stress was a continual problem for the birds suffering from the toxin as they were not able to thermoregulate properly in many cases. A higher metabolic rate would only exacerbate this problem. DDT exposure in mallards (*Anas platyrhynchos*) resulted in higher mortality when challenged with duck hepatitis virus (Peterle 1991). Selenium has also

been investigated for its immune suppression potential. Elevated consumption of selenomethionine was found to be associated with suppression of the delayed-type hypersensitivity to disease challenge in mallards (Fairbrother and Fowles 1990). Selenomethionine was also found to induce glutathione peroxidase and alanine aminotransferase in these birds, the latter being indicative of hepatotoxicity. While this indicates that selenium exposure could be factor in susceptibility to pathogenic organisms, it is not clear that this immune suppression would influence the susceptibility to avian botulism toxin.

## **Methods**

Specimen birds were collected randomly from the dead birds being brought into the Salton Sea National Wildlife Refuge for incineration. The brown pelicans were set aside in advance of collection and frozen until the tissues were collected. One of those birds had been sampled for liver tissue for disease studies by the National Wildlife Health Center, so the remaining liver tissue was collected for the sample analyzed here. The white pelicans that were used had been picked up from the field on the day the samples were collected. All samples were collected with clean instruments and placed in chemically clean jars. The samples were put on ice for transportation to the field office where they were frozen until shipment to the analytical laboratory.

All breast muscle samples were collected from the right side of the specimen birds. Samples of approximately 40-80 g were collected from the brown pelicans, and samples from the white pelicans were approximately 80-110 g. Efforts were made to collect the central mass of muscle; peripheral portions may not have been collected. The samples were weighed at the time of collection. Muscle samples were collected for analysis of organic constituents.

Liver samples were collected from the same birds as the muscle samples. Six additional brown pelican liver samples (for which there are no corresponding muscle samples) were collected one month earlier by the National Wildlife Health Center and provided to this office. Samples were generally comprised of a single lobe of the liver provided it appeared to be adequate mass for the required analyses. Brown pelican liver samples were generally 15-60 g, and white pelicans samples were 40-90 g. The samples were weighed at the time of collection, and any abnormalities in the appearance of tissue were noted. Liver samples were collected for inorganic constituents.

Brain tissues were collected from the brown pelicans for cholinesterase measurements. This was done as an initial screen to determine the potential for impacts from organophosphate and carbamate pesticides. Tissues were collected by removing the upper portion of the skull. Approximately 15-18 g of tissue was collected for analysis, and the samples were weighed at the time of collection.

Chemical analyses on the muscle (organochlorine compounds) and liver (inorganics) tissue were

conducted by Hazelton Environmental Services, Inc. Patuxent Research Center provided the cholinesterase determinations. Analytical methods are provided in the Appendix.

Geometric means were determined for the concentration data for each species separately. Means were only calculated if non-detect results did not exceed half of the samples. One half of the detection limit was used in the calculation of the mean in those cases. Statistical analyses comparing the two species were conducted only on analytes that may have contributed to the die-off event or where a pattern was suspected in the data. A non-parametric statistic was used because of the small samples size and high variance in the data set. A Kruskal-Wallis one-way analysis of ranks test was conducted using Statgraphics version 5.0. A simple regression test was used to evaluate the correlation between the iron and mercury results. A level of  $P < 0.05$  was considered statistically significant.

## **Results**

Detection frequencies, geometric means and ranges are provided in Table 1 for organochlorine compounds and in Table 2 for inorganic elements. The data are provided separately for the two species.

PCB s, dieldrin, p,p DDE, arsenic, boron, cadmium, iron, mercury, and selenium concentrations were compared between the two species. We also look at the correlation between iron and mercury because there were similar patterns in the data between the two. Significant differences between the two species were found for iron and mercury (white pelicans were higher) and cadmium (brown pelicans were higher). No other statistically significant differences were found between the two species. There was a statistically significant correlation between iron and mercury with an  $r^2$  value of 59%, suggesting some link between these two analytes.

## **Discussion - Organics**

Most organochlorine compounds were detected in no or only a few samples. Because of the low detection frequencies and concentrations, the following compounds are not considered to be of importance relative to the die-off event: alpha-BHC, alpha-chlordane, beta-BHC, endrin, gamma-BHC, gamma-chlordane, HCB, heptachlor epoxide, mirex, oxychlordane, toxaphene, and trans-nonochlor. Summary statistics are provided for those compounds in Table 1, and will not be included in the discussion.

### Dieldrin

This analyte was detected in nine of ten brown pelican muscle samples at concentrations up to 0.44 g/g wet weight (WW), and nine of ten white pelican muscle samples had detectable dieldrin at concentrations up to 0.15 g/g WW (Table 1). These concentrations are similar to or

below concentrations measured in carcasses in experimental studies on reproduction where no measurable effects were detected (Peakall 1996). The concentrations we measured were two to three orders of magnitude below liver concentrations in birds which had died of dieldrin exposure (Peakall 1996). The mean values here did exceed most of the mean values for northern pintail (*Anas acuta*) wings measured in California by Ohlendorf and Miller (1984), but the means and maximums they measured for male and female northern shoveler (*Anas clypeata*) carcasses were very similar to the mean and maximum we measured in white pelicans. King and Krynitsky (1986) considered the carcass concentrations they found in waterbirds of Galveston Bay to be low as they were all below 1 g/g. All of the concentrations measured here were below 1 g/g, including the highest concentration of 0.44 g/g WW measured in a brown pelican sample. It is not clear why there is a slight difference between brown pelicans and white pelicans, but it does not appear to be significant relative to the die-off.

### Polychlorinated Biphenyls (PCB s)

This area is not subject to large volumes of industrial waste, so screening for total PCB s was adequate for our purposes. Maximum concentrations of total PCB s in brown and white pelican muscle samples were 0.69 and 2.0 g/g WW, respectively (Table 1). The mean concentrations for brown and white pelicans were 0.24 and 0.34 g/g WW, respectively. These concentrations are similar to carcass concentrations measured for clapper rails (*Rallus longirostris*) at Cape May, New Jersey in 1973 (Klaas and Belisle 1977) which were considered to be low in that study. Ohlendorf and Miller (1984) found a pooled concentration of 0.34 g/g WW in wings of male and female canvasbacks (*Aythya valisineria*) wintering at the Salton Sea. This is the same as the mean for the white pelican samples in this study. The highest pooled concentration in the Ohlendorf and Miller (1984) study was for male canvasbacks (wings) in San Francisco Bay in winter at 1.6 g/g WW. Lesser scaup (*Aythya affinis*) from the Salton Sea had a pooled wing concentration of 0.22 g/g WW in that study, which was very close to the mean we measured in brown pelicans. In contrast, olivaceous cormorants (*Phalacrocorax olivaceus*) another piscivorous species from Galveston Bay had a mean carcass concentration of 7.6 and a maximum of 24 g/g WW in 1981 (King and Krynitsky 1986). The authors of that study considered the concentrations they had measured to be below levels associated with chronic poisoning and reproductive problems. Heinz *et al.* (1984) also found similar concentrations to those in Galveston Bay in great blue herons (*Ardea herodias*) along the Sheboygan River in Wisconsin. Even higher concentrations (up to 218 g/g WW) were found in belted kingfishers (*Ceryle alcyon*) in that study. These data suggest that PCB s are not of major concern in the Salton Sea ecosystem as compared to more industrialized areas along the Great Lakes and the Gulf Coast.

### Dichlorodiphenyltrichloroethane and its Metabolites (DDT s)

Six DDT compounds were included in the analysis of the muscle samples. o,p DDD was not detected in any of the samples (Table 1). o,p DDT was detected in one of the brown pelican samples at a concentration of 0.01 g/g WW. p,p DDT was detected in two brown pelican samples at 0.013 and 0.017 g/g WW. It was also detected in two white pelican samples at

0.011 and 0.012 g/g WW. While the concentrations of the latter two analytes are not significant in terms of impacts, it is significant that parent DDT compounds are still being detected in addition to the DDD and DDE breakdown products. This suggests that either the half-lives of the parent compounds in soils are longer than the 10-20 years believed to be the case (USEPA 1975), or that the birds are exposed to more recently applied material somewhere along their migratory route.

o,p DDE was found in almost half of the muscle samples analyzed. Four of the ten brown pelicans had concentrations up to 0.043 g/g WW. Five of the ten white pelicans had concentrations up to 0.034 g/g WW. These concentrations represent a small percentage of the total DDT exposure. This particular compound has not been related to adverse effects (Blus 1996). Therefore, this compound does not appear to pose a risk to these two populations and was not likely to have been a factor in the die-off.

p,p DDD was found in the majority of the muscle samples (nine of ten in brown pelicans and all ten white pelicans). The concentrations of p,p DDD measured were also at relatively low levels for both the brown pelicans (maximum of 0.13 g/g WW) and the white pelicans (maximum of 0.20 g/g WW). These concentrations would only contribute slightly to the impacts associated with this group of compounds and are not likely to have been an independent factor in the die-off.

The p,p DDE residues measured in the muscle tissue comprised the largest proportion of the total DDT residues measured. The brown pelican samples had a mean of 2.6 g/g WW and a range of 0.13-10 g/g WW. For white pelicans the concentrations were slightly lower with a mean of 1.5 g/g WW and a range of 0.45-4.3 g/g WW. These results (particularly those for brown pelicans) are similar to carcass concentrations found in clapper rails in New Jersey in 1967 (Klaas and Belisle 1977) and olivaceous cormorants, black skimmers and laughing gulls in Galveston Bay in 1980-81 (King and Krynitsky 1986). The levels measured here are of concern because the concentrations (measured in 1996) are so similar to concentrations measured while DDT was still in use or less than ten years after the ban on domestic use in 1972. It is possible that these birds are being exposed to DDT in Mexico, as both species are known to migrate to Mexico for part of the year. However, it is not known what proportion of these two populations remains at the Salton Sea and how many move on into Mexico. In addition, Mora (1997) found that DDE concentrations in birds from the southwestern United States were comparable to those in Mexico suggesting that applications south of the border may not be the only source of DDE found in these samples. Because DDE has been detected in the majority of biota samples collected at and around the Salton Sea (Setmire *et al.* 1993), we have to consider that the DDE measured was accumulated in the Salton Sea ecosystem. Ohlendorf and Miller (1984) found increases in pintail wing concentrations of DDE during the winter in the Imperial Valley, suggesting a local source.

King and Krynitsky (1986) identified the carcass levels they found (which were similar to those measured here) as being below levels associated with chronic poisoning and reproductive

problems in most species. Henny *et al.* (1990) also found DDE in western grebes (*Aechmophorus occidentalis*) in Commencement Bay, Washington at concentrations of 0.45- 7.0 g/g. They did not find any indication of toxicological significance in that the birds appeared to be in good condition. As the concentrations measured here were similar to those two studies, it would not appear that p,p DDE concentrations contributed significantly to the die-off event. Additional studies of these populations would be required to determine if DDE is accumulating in the eggs and impacting reproduction. This is of concern because of the low threshold for reproductive effects of 3 g/g DDE in the egg seen in brown pelicans by Blus (1982).

## **Inorganics**

### Aluminum

Scheuhammer (1987) described the direct toxic potential of aluminum as being low compared to that of many other metals. Aluminum was detected in four of the sixteen brown pelican liver samples (range <1.61-18.8 g/g Dry Weight (DW); Table 2) and one of the ten white pelican liver samples (4.13 g/g DW). These liver concentrations are similar to those found in surf scoters (*Melanitta perspicillata*) from San Francisco Bay (Ohlendorf *et al.* 1991) and were well below the maximum of 120 g/g DW measured in canvasbacks from San Francisco Bay (Miles and Ohlendorf 1993). The values measured here are largely within the control values in liver tissue provided by Sparling and Lowe (1996). Those authors suggest that the birds are most susceptible to aluminum toxicity as rapidly growing chicks, with tolerance to aluminum increasing as chicks approach adult size. All birds collected in this study, and the vast majority of the birds affected by the die-off, were essentially adult size. Therefore, aluminum is not likely to have contributed to the die-off event.

### Arsenic

Arsenic was detected in all 16 of the brown pelican and all ten of the white pelican liver samples. The maximum in brown pelican livers (2.87 g/g DW; Table 2) was slightly higher than that for white pelicans (2.32 g/g DW), but the geometric mean for the white pelicans was higher than for brown pelicans (1.53 and 1.05 g/g DW, respectively). The concentrations measured here were within the range of bird liver background concentrations provided by Eisler (1994). Dietary studies summarized by Eisler (1994) showed no ill effects at three to ten times the liver concentrations measured here.

Other studies which evaluated arsenic showed similar concentrations to those found here. Miles and Ohlendorf (1993) determined arsenic liver concentrations in canvasbacks in San Francisco Bay of the same order of magnitude as those found here ( =0.25 g/g DW, maximum=1.1 g/g DW). Ohlendorf *et al.* (1991) also found similar liver concentrations in surf scoters in San Francisco Bay (means were 0.46 to 1.25 g/g DW), which they considered to be background levels. Therefore, arsenic contamination in these birds is not likely to have contributed significantly to the die-off.

## Barium

Barium was detected in four of the 16 brown pelican liver samples but none of the white pelican livers. The detected values were 3.96-38.8 g/g DW (Table 2), with three of the four in the 4-5 g/g DW range. The reason for the exceptional value over 38 g/g DW is not known. For comparison, San Diego Bay brown pelicans had liver concentrations of 0.23-0.51 g/g DW (USFWS unpublished data). There is little information available on the significance of barium tissue concentrations in wildlife, likely because it is rarely detected. While the source of the single high value detected here calls for further investigation, barium is not likely to be a contributing factor in the die-off due to the low frequency of detection, particularly in the white pelicans which were most greatly affected in the die-off.

## Beryllium

Beryllium was not detected in any of the pelican liver samples (Table 2) and will not be considered further.

## Boron

Boron was detected in nine of the 16 brown pelican liver samples and nine of the ten white pelican samples (Table 2). The maximum in brown pelicans (5.92 g/g DW) was higher than for white pelicans (3.63 g/g DW), but the geometric mean in white pelicans (2.16 g/g DW) was slightly higher than that for brown pelicans (1.41 g/g DW). San Diego Bay brown pelicans had comparable concentrations at 0.69 to 4.1 g/g DW (USFWS unpublished data). These concentrations are below those found in adult mallards which displayed reproductive problems as a result of boron supplementation of the diet (Smith and Anders 1989). Reduced chick growth did occur even at the lowest adult supplementation rates where the adult liver concentrations were similar to the concentrations measured here. Their study did indicate that the adverse effects of boron occurred in the egg in the form of increased embryo mortality with no long-term toxicological effects to the adults being noted. Hoffman *et al.* (1991) found reduced duckling growth as a result of boron in the diet, particularly in combination with selenium supplements and reduced dietary protein. However, boron alone did not cause mortality except at the highest dietary levels or result in histopathological lesions in the organs examined. While further investigation would be required to determine if boron exposure is causing reproductive problems for these birds, it does not appear to be a factor in the die-off of juvenile and adult pelicans at the Salton Sea.

## Cadmium

Cadmium was detected in all 16 of the brown pelican liver samples and six of the ten white pelican samples (Table 2). The brown pelican samples had a higher geometric mean (2.94 g/g DW) and maximum (11.3 g/g DW) than the white pelican samples (0.35 and 1.87 g/g DW, respectively). There was a statistical difference between the two species, although the cause of

this difference could not be identified. These concentrations are similar to, and in some cases well below, residues from field collections reported by Eisler (1985). Furness (1996) provides a summary of cadmium concentrations in bird populations considered to be healthy. Most of those concentrations were greater than those measured here, including those from areas where cadmium contamination was considered negligible. Furness (1996) identified 40 g/g WW in the liver as a tentative threshold value. The maximum concentration measured here as a wet weight was 3.24 g/g.

This study found liver concentrations similar to those in surf scoters in San Francisco Bay (Ohlendorf *et al.* 1991). The liver concentrations measured in white pelicans were similar to canvasbacks from San Francisco Bay (Miles and Ohlendorf 1993), but the brown pelicans sampled here had slightly higher mean and maximum concentrations than in that study. Scheuhammer (1987) considered 3 g/g DW to be background for waterfowl liver concentrations. The geometric means for both species sampled here were below that level, and all were below the tentative threshold identified by Furness (1996). Cadmium does not appear to have contributed significantly to the die-off of these two species at the Salton Sea.

### Chromium

Chromium was detected in all 16 of the brown pelican and all ten of the white pelican liver samples (Table 2). The maximum in brown pelicans (2.11 g/g DW) was slightly higher than that for white pelicans (1.97 g/g DW), but the geometric mean was higher in white pelicans (0.85 g/g DW) than in the brown pelicans (0.69 g/g DW). San Diego Bay brown pelicans had similar liver concentrations of chromium at 1.39-2.62 g/g DW (USFWS unpublished data). Eisler (1986) identifies a tissue concentration of chromium of greater than 4 g/g DW as indicative of chromium contamination.

In San Francisco Bay canvasbacks chromium was detected in 45% of the liver samples tested (Miles and Ohlendorf 1993). However, the maximum concentration (13 g/g DW) measured in that study was higher than concentrations measured here indicating that these values are not exceptional. The concentrations of chromium measured here do not appear to be elevated, and it is therefore unlikely that chromium contamination was a factor in the die-off event at the Salton Sea.

### Copper

Copper was detected in all brown and white pelican liver samples (Table 2). The ranges and geometric means for the two were similar, with the white pelicans ( $\bar{x}$ =15.7 g/g DW, maximum=38.9 g/g DW) having slightly higher values than the brown pelicans ( $\bar{x}$ =13.7 g/g DW, maximum=33.3 g/g DW). These values were below the corresponding concentrations for brown pelicans in San Diego Bay ( $\bar{x}$ =37.3 g/g DW, maximum=96 g/g DW; USFWS unpublished data), a site with known copper contamination.

The liver concentrations measured in this study were below concentrations measured in surf scoters in San Francisco Bay (Ohlendorf *et al.* 1991), which were not believed to represent a hazard to that species. Copper concentrations in livers of canvasbacks in San Francisco Bay (Miles and Ohlendorf 1993) were much higher than those reported here, as were those in canvasbacks from Catahoula Lake, Louisiana (Custer and Hohman 1994). The levels detected in the former study ( =99 g/g DW, maximum=600 g/g DW) were identified as being indicative of low level exposure, and copper was considered an innocuous trace element. It appears that a wide range of tissue concentrations of this element is tolerated in birds. Because the concentrations measured here are low relative to other areas, copper does not appear to be of concern in the die-off of these species at the Salton Sea.

### Iron

Iron was detected in all liver samples analyzed (Table 2). As an essential nutrient, iron is not frequently considered a potential contaminant. The geometric mean and maximum were higher in white pelicans (4140 and 10,200 g/g DW, respectively) than in brown pelicans ( =2010 g/g DW, maximum=4690 g/g DW). This difference was statistically significant. Brown pelicans from San Diego Bay had liver concentrations of iron that were very similar to those collected at the Salton Sea (USFWS unpublished data). These liver concentrations did exceed by two to six times those found in surf scoters and canvasbacks in San Francisco Bay (Ohlendorf *et al.* 1991, Miles and Ohlendorf 1993). Miles and Ohlendorf (1993) considered iron to be an innocuous trace element, suggesting that it was unlikely to have contributed to the die-off. The significance of the extremely high values in some of the white pelicans does warrant future investigation, however. Because there was a correlation with mercury, it is possible that the sources of these two constituents may be related.

### Lead

Lead was not detected in any of the pelican liver samples (Table 2) and will not be considered further.

### Magnesium

Magnesium was detected in all pelican liver samples (Table 2). The geometric mean and maximum concentrations for white pelicans (604 and 749 g/g DW, respectively) were only very slightly higher than those for brown pelicans (576 and 741 g/g DW, respectively). San Diego Bay brown pelicans had liver concentrations in the same range ( =608 g/g DW, maximum=741 g/g DW; USFWS unpublished data). These are very similar to concentrations found in canvasback livers from Catahoula Lake, Louisiana (Custer and Hohman 1994). These levels would appear to be normal and are not likely to have contributed to the die-off event.

### Manganese

Manganese was detected in all of the brown and white pelican liver samples analyzed in this study (Table 2). The concentrations were similar between the two species with brown pelicans having slightly higher concentrations (  $\bar{x}$ =9.01 g/g DW, maximum=13.4 g/g DW) than white pelicans (8.55 and 11.3 g/g DW, respectively). San Diego Bay brown pelicans had somewhat higher concentrations with a geometric mean of 12.5 g/g DW and maximum of 22.1 g/g DW (USFWS unpublished data). Canvasbacks from Catahoula Lake, Louisiana had similar, but slightly higher (10.2-14.6 g/g DW), liver concentrations (Custer and Hohman 1994). Surf scoters in San Francisco Bay had similar concentrations to those in the San Diego Bay pelicans (15-21 g/g DW; Ohlendorf *et al.* 1991). The liver concentrations found in that study were not expected to result in observable effects. Because the liver concentrations detected here are even lower, manganese is not expected to have been a factor in the die-off event.

### Mercury

Mercury was detected in 15 of the 16 brown pelican liver samples and all ten of the white pelican samples (Table 2). The brown pelican samples had much lower mercury concentrations (  $\bar{x}$ =0.40 g/g DW, maximum=3.11 g/g DW) than the white pelicans (10.6 and 104 g/g DW, respectively). The difference between the two species was statistically significant. The brown pelican samples had liver concentrations which are fairly typical of concentrations found in many areas (Eisler 1987). Although not as high as concentrations found in heavily contaminated areas (Eisler 1987), the white pelicans had liver concentrations which were clearly elevated above what could be considered background. In fact, these concentrations are similar to the liver residues associated with birds experimentally given lethal doses of methyl mercury (17 to 70 g/g DW; Eisler 1987). Thompson (1996) concluded that mercury concentrations in the liver in excess of 30 g/g WW appear lethal to a variety of birds of prey. One of the white pelican liver samples exceeded this level, although the other samples were in the range of 1-4 g/g WW. The liver concentrations we measured were similar to liver concentrations found in mercury feeding studies of several bird species (Thompson 1996) supporting that the concentrations found in white pelicans in this study are not background. Exposure may be tied to an iron source for the white pelicans as there was a correlation between the two.

Several other studies have looked at mercury contamination in birds. Mean liver concentrations for greater scaup (*Aythya marila*) and surf scoters from San Francisco Bay were 10.6 and 12.5 g/g DW, respectively (Ohlendorf and Fleming 1988). These levels were identified as being high enough to have the potential for effects. Canvasbacks showed lower mercury levels with a mean of 3.1 g/g DW (Miles and Ohlendorf 1993) which were considered to be at background. Western grebes (*Aechmophorus occidentalis*) from Commencement Bay, Washington, a mercury enriched area, had liver mercury concentrations early and late in the wintering period of 1.90 and 2.48 g/g DW, respectively (Henny *et al.* 1990). These birds appeared to be healthy at the time of collections, although sublethal effects were not evaluated. Ohlendorf (1993) identified background levels of mercury in the liver to be 1-10 g/g DW, but also identified concentrations greater than 6 g/g DW to be toxic. Eight of the ten white pelican liver samples analyzed here exceeded that threshold. While it is not clear what type of impairment may have resulted from

these levels, further examination of mercury contamination should be pursued in this white pelican population. Future studies should evaluate brain tissue histopathology in combination with residue analysis. In addition, this population may be subject to reproductive impairment which would increase the impacts of such die-off events on the population as a whole.

Interactive effects can occur between mercury and selenium (Eisler 1987, Scheuhammer 1987, Ohlendorf *et al.* 1991, Ohlendorf 1993, and Heinz 1996). Scheuhammer (1987) suggests that by association with high levels of selenium, mercury can accumulate in liver tissues in mammals without exerting any apparent toxicity. This would be important in the white pelicans considering the elevated mercury levels found, but similar correlations between mercury and selenium have not been established in birds. In fact, positive, negative and no correlations have all been found between the two elements across an array of bird species (Ohlendorf *et al.* 1991). This may be related to migration patterns and different bioaccumulation times between the two elements. Such a protective effect may only be reached at higher mercury levels long after the arrival in a mercury contaminated area (Scheuhammer 1987).

Mercury toxicity can also be increased by pesticides such as DDE and elevated temperatures (Eisler 1987). While DDE is bioavailable in this ecosystem, the residues detected here were relatively low. Temperatures were definitely elevated at the time of the die-off, exceeding 100°F on a daily basis. However, the birds displayed classic symptoms of botulism intoxication and did not display symptoms of mercury poisoning (Tonie Rocke, pers. comm.). While additional study is warranted on the effects of mercury contamination in this population, mercury does not appear to have been a major causative factor in the die-off event.

### Molybdenum

Molybdenum was detected in ten of 16 brown pelican and all ten white pelican liver samples (Table 2). The white pelicans had slightly higher geometric mean and maximum (2.23 and 3.08 g/g DW, respectively) concentrations than the brown pelican samples (1.25 and 2.5 g/g DW, respectively). Brown pelicans from San Diego Bay had similar concentrations with a geometric mean of 1.85 g/g DW and a maximum of 2.80 g/g DW (USFWS unpublished data). Values below 4 g/g DW are considered low by Eisler (1989). This suggests that molybdenum did not significantly contribute to the die-off event.

### Nickel

Nickel was only detected in one liver sample from each species (Table 2). The liver concentrations detected (0.59 and 0.92 g/g DW for brown and white pelicans, respectively) were below concentrations found in ducklings that died during an experimental exposure to nickel in the diet (Cain and Pafford 1981). These concentrations were also below the single liver concentration of 2 g/g DW detected among canvasbacks from Catahoula Lake, Louisiana (Custer and Hohman 1994). As a result of the infrequent detection and the low detected concentrations, this element is not considered significant to the die-off event.

## Selenium

Selenium was detected in all brown and white pelican liver samples (Table 2). The geometric mean in white pelicans (12.5 g/g DW) was slightly higher than that for brown pelicans (11.5 g/g DW), but the maximum was higher in the brown pelicans (35.4 g/g DW) than in the white pelicans (20.9 g/g DW). Heinz (1996) identified three liver residue thresholds: 3 g/g WW is the threshold associated with reproductive impairment, 10 g/g WW is associated with significant sublethal effects, and above 20 g/g WW survival is jeopardized. All samples in this sample set had wet weight concentrations below 10 g/g. However, 11 of the 16 brown pelicans and seven of the ten white pelicans exceeded the reproductive impairment threshold which is worthy of future investigation. Species that prefer more saline environments do appear to have a higher selenium tolerance (Skorupa, J.S., S.P. Morman, and J.S. Sefchick-Edwards, Guidelines for Interpreting Selenium Exposures of Biota Associated with Non-marine Aquatic Habitats, prepared for the National Irrigation Water Quality Program, 1996). Brown pelicans are marine birds, and white pelicans frequently use coastal areas and alkali lakes and so may have a greater tolerance for selenium. The Guidelines identify the hepatic threshold for juvenile and adult toxicity at 30 g/g DW. One of the brown pelican samples did exceed that threshold, but the significance is unclear as the tolerance for selenium is not well understood in this species.

Selenium was found in canvasback livers from San Francisco Bay (Miles and Ohlendorf 1993) at slightly higher levels than those found here. The levels found were within the range associated with impaired reproduction in waterfowl. The significance in wintering birds is unclear because of the rapid decline in liver concentrations after the selenium is removed from the diet (Heinz *et al.* 1990). San Francisco Bay greater scaups and surf scoters had liver residues of 19.3 and 34.4 g/g DW, respectively (Ohlendorf and Fleming 1988). The Chesapeake Bay birds considered in that study did not have detectable concentrations of selenium, and the levels in the San Francisco Bay ducks were similar to ducks from the San Joaquin Valley where reproduction was found to be impaired. Some of the concentrations from San Francisco Bay were as high as those found in coots which had died of selenium toxicosis at Kesterson National Wildlife Refuge.

Shorebirds in Washington (Ohlendorf 1993) had similar median liver concentrations to the means found here. The concentrations for dunlins (*Calidris alpina*), long-billed dowitchers (*Limnodromus scolopaceus*), and black-bellied plovers (*Pluvialis squatarola*) were 13, 11, and 9.4 g/g DW, respectively. The two highest concentrations of 27 and 30 g/g DW were both found in plovers. Ohlendorf (1993) identified these high values as concentrations where sublethal effects on bird health could be occurring, but no specific toxic effects of selenium have been identified in marine birds.

Selenium concentrations and effects can also be modified by the presence of other trace elements. Hoffman *et al.* (1991) found that several physiological parameters in mallard ducklings were subject to greater impacts as a result of a combination of boron and selenium in the diet, particularly if protein consumption was low. On the other hand, Hoffman *et al.* (1992) found the arsenic alleviated some of the effects of elevated selenium in the diet, but less

effectively with reduced protein intake. It is not clear what these interactive effects would mean for juvenile or adult birds which have attained their full body size. Both boron and arsenic are bioavailable in this ecosystem, but the relative magnitude of these opposing effects is not understood for these species.

The available information does not clearly indicate whether the selenium concentrations found are problematic for pelicans or not. Other species of aquatic birds would likely be impacted by the concentrations found here, particularly in terms of reproductive effects. Tolerance does vary among species, however, so no clear conclusion can be drawn regarding the likelihood of impact in these two pelican species. Pending more specific tolerance data, we can only conclude that selenium contamination may have been a contributing factor in the overall health and susceptibility to the botulism toxin of the birds which succumbed during the die-off.

### Strontium

Strontium was detected in 14 of the 16 brown pelican liver samples and all ten of the white pelican liver samples (Table 2). The concentrations were similar for the brown and white pelicans with geometric means of 0.44 and 0.45 g/g DW, respectively. The maximum was higher for the brown pelicans samples at 3.71 g/g DW versus 1.57 g/g DW for the white pelican samples. Little data is available in the literature for strontium. The geometric mean concentration in San Diego Bay brown pelicans was 0.27 g/g DW, with a maximum of 0.54 g/g DW (USFWS unpublished data). While the significance of these residues cannot be determined based on comparison with literature values, overall the concentrations were low (with means below 1 g/g DW) and are not likely to have played a major role in the die-off.

### Vanadium

Vanadium was detected in 12 of 16 brown pelican and all ten white pelican liver samples (Table 2). White pelicans had slightly higher geometric mean and maximum (0.71 and 1.25 g/g DW, respectively) concentrations than did the brown pelican samples (0.23 and 0.62 g/g DW, respectively). This analyte is rarely discussed in the literature. It was not detected in brown pelicans from San Diego Bay (USFWS unpublished data). However, the concentrations found were below those found in wintering shorebirds from Corpus Christi, Texas (R. Eisler, pers. comm.). Custer and Hohman (1994) detected vanadium in canvasback livers from Catahoula Lake, Louisiana at concentrations up to 0.94 g/g DW. As with strontium, no specific potential impacts of these vanadium concentrations could be identified. Because the concentrations were again low (only one sample exceeded 1 g/g DW), it is unlikely that this trace element was significant in the die-off.

### Zinc

Zinc is an essential trace element so its detection in all of the pelican liver samples (Table 2) was expected. White pelicans had slightly higher concentrations ( =138 g/g DW, maximum=295

g/g DW) than the brown pelicans collected ( =125 g/g DW, maximum=282 g/g DW). Brown pelicans from San Diego Bay had somewhat higher concentrations with a geometric mean of 229 g/g DW and a maximum of 529 g/g DW (USFWS unpublished data). Eisler (1997) identified normal zinc concentrations in marine birds of up to 88 g/g WW in the liver. Only one white pelican liver sample collected here exceeded that value (at 98 g/g WW). Mallards fed zinc supplements had liver concentrations which were much higher than the concentrations measured here (Gasaway and Buss 1972). In fact, the mean liver concentrations measured here were below that for controls in that feeding study.

Mean liver values for greater scaup and surf scoters in San Francisco Bay were similar to the concentrations measured here (151 and 131 g/g DW, respectively; Ohlendorf and Fleming 1988). Canvasbacks in San Francisco Bay also had similar liver concentrations with a mean of 160 g/g DW, which were similar to canvasbacks from an uncontaminated area (Miles and Ohlendorf 1993). Custer and Hohman (1994) had mean liver concentrations of 116-147 g/g DW in canvasbacks from Catahoula Lake, Louisiana. This would indicate that the concentrations found here are fairly typical for birds in salt-water environments. Therefore, zinc is not likely to have contributed to the die-off event.

### **Acetylcholinesterase**

Brain cholinesterase activity measurements were taken on brain samples from the ten brown pelicans from which liver and muscle samples were collected. Comparative data for normal pelican cholinesterase values are not readily available in the literature. However, the acetylcholinesterase assay included a reactivation step. This reactivation step exposes the tissues to pyridine-2-aldoxime methochloride (2-PAM) which can remove bound organophosphates and thus restore cholinesterase activity (Fairbrother *et al.* 1991). Five of the ten brown pelican samples tested showed reactivation indicative of organophosphate exposure. Acute exposure to cholinesterase inhibiting pesticides such as organophosphates can result in incoordination, muscular weakness and slight paralysis in birds (Grue *et al.* 1991). The symptoms of botulism intoxication are similar, although more specific, and include paralysis of the neck muscles and inability to fly (Locke and Friend 1987).

Thermoregulatory impacts are also known to occur with exposure to cholinesterase inhibiting pesticides, including increased inhibition at high temperatures (Grue *et al.* 1991). The high temperatures which accompanied the die-off may have exacerbated the impacts of pesticide exposures and compounded the difficulties in thermoregulation experience by birds exposed to the botulism toxin. However, the botulism toxin is lethal in nanogram quantities thus minimizing the effects of organophosphate pesticide exposure relative to the effects of the toxin, as demonstrated by the fact that the birds displayed symptoms consistent with botulism intoxication and not organophosphate pesticide poisoning (Tonie Rocke, pers. comm.).

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## Appendix

LABORATORY: Hazleton Environmental Services, Inc.

### Extraction by Soxhlet

#### SCOPE:

This method covers the extraction of Organochlorine Pesticides and Polychlorinated Biphenyls (PCBs) in biological tissues.

#### SAMPLE PREPARATION:

Blend 20 grams of ground tissue with 40 grams of anhydrous sodium sulfate in a 250 ml beaker. If there is not 20 grams of sample available the remove at least one gram for percent moistures and weigh the remainder for extracting. For wet samples, more sodium sulfate may be required. If a sufficient amount has been added the sample will appear granular. Add 1.0 ml of the pesticide spiking solution to the matrix spike and the control spike. Add 200 ul of the 2,4,5,6-tetrachloro-m-xylene (TMX) surrogate spiking solution to all samples and QC samples. Allow the ground tissue/sodium sulfate to dry under a hood for a couple of hours, stirring it occasionally.

#### PROCEDURE:

Load the prepared sample into the soxhlet extractor between two plugs of pre-extracted glass wool. Place 250 ml of methylene chloride into a pre-rinsed 500 ml erlenmeyer flask containing three to five teflon boiling chips. Attach the flask to the extractor. Add 100 ml of methylene chloride to the mixing beaker, swirl, and add the solvent to the extractor prior to attaching the condenser. Adjust the temperature of the heating mantle so that the extractors cycle at a rate of 12 to 15 cycles per hour. Allow the system to cycle for 16 to 20 hours.

Allow the extract to cool after the extraction is complete. Rinse the condenser with extraction solvent and drain the soxhlet apparatus into the bottom collection flask.

Pour the extract through a Whatman #4 filter into a 500 ml K-D flask fitted with a 10 ml concentrator tube. Attach a three-ball snyder column to the K-D flask and concentrate the extract on a hot water bath,

adjusting the temperature so that the concentration is completed within 15 to 20 minutes.

When the apparent volume reaches approximately 5.0 ml, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Bring up to a volume of 10 ml with methylene chloride.

REFERENCES:

1. Environmental Protection Agency, "Test Methods for Evaluating Solid Waste - Physical/Chemical Methods - EPA Publication No. SW-846," Method 3540, Office of Solid Waste and Emergency Response, Washington, D.C. (September 1986)
2. "Determination of Organochlorine Pesticides and Polychlorinated Biphenyls (PCBs) in Biological Tissues." Method MP-FWST-MA, Hazleton Environmental Services, Inc., Madison, Wisconsin.

Determination of Percent Lipids

SCOPE:

This method covers the gravimetric determination of percent lipids in biological tissue samples.

PROCEDURE:

One milliliter of the 10 ml extract is placed into a preweighed aluminum weighing pan. The pan is allowed to sit lightly covered in a hood overnight to allow the solvent to evaporate. The pan is then weighed again. The following equation is then used to calculate the percent lipid:

$$\frac{((\text{weight(g) of pan + lipid}) - \text{weight(g) of pan}) \times 10 \text{ ml} \times 100}{\text{grams extracted}} = \% \text{lipid}$$

REFERENCES:

1. "Determination of Organochlorine Pesticides and Polychlorinated Biphenyls (PCBs) in Biological Tissues." Method MP-FWST-MA, Hazleton Environmental Services, Inc., Madison, Wisconsin.

## Determination of Percent Moisture

### SCOPE:

This method covers the gravimetric determination of percent moisture in soil, sediment and biological tissue samples.

### PROCEDURE:

One to 10 g of the sample is placed into a preweighed aluminum weighing pan. The pan is weighed again with the sample in it. The pan and sample are then placed into an oven at 105 C for 16 hours. The sample is allowed to cool in a desiccator and then weighed again. The following equation is used to calculate the percent moisture:

$$\frac{((\text{mass(g) pan} + \text{wet sample}) - (\text{mass(g) pan} + \text{dry sample})) \times 100}{\% \text{ moisture grams of sample}}$$

If samples are to be calculated based on dry weight, the percent moisture is converted to a correction factor (M). The calculation of the factor is:

$$100 / (100 - \% \text{ moisture}) = M$$

### REFERENCES:

1. Environmental Protection Agency, "Test Methods for Evaluating Solid Waste - Physical/Chemical Methods - EPA Publication No. SW-846," Method 3550, Office of Solid Waste and Emergency Response, Washington, D.C. (September 1986)
2. "Determination of Organochlorine Pesticides and Polychlorinated Biphenyls (PCBs) in Soils and Sediments." Method MP-FWSS-MA, Hazleton Environmental Services, Inc., Madison, Wisconsin.

## Gel-Permeation Chromatography Cleanup

### SCOPE:

This method covers the cleanup of soil, sediment and biological extracts by gel-permeation chromatography (GPC).

### PROCEDURE:

After extraction, the sample extracts are concentrated in Kuderna-Danish (K-D) apparatus and the volume is adjusted to 10 ml with methylene chloride. Five milliliters of this extract is then injected on an ABC Laboratories Model 1002B GPC system using a column packed with 70 g of S-X3 Bio-beads and methylene chloride as the carrier solvent. A dump, collect, and rinse cycle is then run which is consistent with exhibit D, section 7.1 of reference 1 below.

The collected fraction is then quantitatively transferred to a 500 ml K-D apparatus fitted with a 10 ml concentrator tube. A three ball snyder column is attached and the extract is concentrated on a hot water bath, adjusting the temperature such that the concentration is completed within 15-20 minutes.

When the apparent volume reaches approximately 5.0 ml, the K-D apparatus is removed from the water bath. 50 ml of hexane is added to the flask and it is returned to the hot water bath and the extract is concentrated to 5.0 ml.

### REFERENCES:

1. USEPA Contract Laboratory Program, "Statement of Work for Organic Analysis, multi-media, multi-concentration", Document number OLM01.0 (March 1990) including revisions OLM01.1 (December 1990) and OLM01.2 (January 1991)
2. "Determination of Organochlorine Pesticides and Polychlorinated Biphenyls (PCBs) in Biological Tissues." Method MP-FWST-MA, Hazleton Laboratories America, Inc., Madison, Wisconsin.
3. Instrument Operating Procedure for Gel-Permeation Chromatograph, Method OP-6004-36, Hazleton Environmental Services, Inc., Madison, Wisconsin.

## Silica Gel Cleanup and Separation

### SCOPE:

This method is applicable to any sample extract in hexane which requires additional cleanup and the separation of polychlorinated biphenyls (PCBs) from many of the organochlorine pesticides.

### SAMPLE PREPARATION:

The sample extract should be at a volume of 5.0 ml in hexane.

### PROCEDURE:

The silica gel (100/200 mesh) is prepared by swirling it in a slurry of 40% acetonitrile and 60% methylene chloride, vacuum filtering, and then rinsing it successively with methylene chloride and hexane. It is then dried at 140 C overnight and deactivated with 0.5% (w/v) distilled water.

Fifteen grams of this silica gel is then slurried in petroleum ether, poured into a chromatography column, and topped with anhydrous sodium sulfate. The sample extracts are then drawn into the top of the column. The first fraction is eluted with 140 ml petroleum ether. The second fraction is eluted with 250 ml of a mixture of 1% acetonitrile, 19% hexane, and 80% methylene chloride (v/v).

The first fraction should include all PCBs, p,p'-DDE, hexachlorobenzene, and mirex. It may also include some portion of p,p'-DDT, o,p'-DDE, o,p'-DDT, and trans-nonachlor. The remaining portion of these 4 pesticides, along with all other organochlorine pesticides, will be found in the second fraction.

Both fractions are then quantitatively transferred to a 500 ml K-D apparatus fitted with a 10 ml concentrator tube. A three ball Snyder column is attached and the extract is concentrated on a hot water bath, adjusting the temperature such that the concentration is completed within 15-20 minutes.

When the apparent volume reaches approximately 5.0 ml, the K-D apparatus is removed from the water bath and allowed to cool for at least 10 minutes. 50 ml of hexane is added to the flask and it is returned to the hot water bath. If the extract was cleaned by gel-

permeation chromatography (GPC), it is concentrated to 5.0 ml. If it did not undergo GPC cleanup then it is concentrated to 10.0 ml.

#### REFERENCES:

1. Technical Operating Procedure, "Silica Gel Cleanup and Separation of Organochlorine Pesticides and PCBs", Method OP-6004-45, Hazleton Environmental Services, Inc., Madison, Wisconsin.

#### Elemental Analysis by Inductively Coupled Plasma Spectroscopy

#### SCOPE:

This method is applicable to plant and animal tissue, soil/sediment, and water.

#### Sample Preparation:

##### 1) Plant and Animal Tissue

- Digest 5.00 g of tissue in Teflon vessel with 5 mL nitric acid in microwave digester. Transfer into 50 mL volumetric flask and dilute to volume with 0.005% Triton X-100 solution. Filter.

##### 2) Soil/Sediment

- Digest 1.00 g in covered Teflon beaker on hot plate using 10 mL nitric acid. Add 30% hydrogen peroxide in 1 mL aliquots until effervescence no longer occurs. Add 1.25 mL hydrochloric acid, heat 10 minutes, and transfer to a 50 mL volumetric flask. Dilute to volume with DDI water. Filter.

##### 3) Water

- Digest 100.0 mL sample in Teflon beaker on hot plate with 0.5 mL nitric acid and 2.5 mL hydrochloric acid. Reduce volume to 15 to 20 mL. Transfer into 50 mL volumetric flask. Dilute to volume with DDI water. Filter.

#### PRINCIPLE:

Each analyte concentration in the sample solution is determined by comparing its emission intensity with the emission intensities of a known

series of analyte standards. The analytical wavelengths are tabulated with the raw concentration data. Analytical data is corrected for background and interfering element effects by the spectrometer program. The detection limit of each analyte is listed in the data report with each respective unknown value, it is a function of the instrument detection limit (IDL), and the sample mass and volume to which it is diluted. With each batch of 20 samples of the same matrix type, at least one duplicate, one sample spike, one analytical blank, and one appropriate reference material are assayed.

#### REFERENCE:

1. Test Methods for Evaluating Solid Waste - EPA Publication No. SW-846, 3rd edition, Methods (3030, 3040, or 3050) and 6010, US EPA, Washington DC (revised December 1987).
2. Dahlquist, R.L. and Knoll, J.W., "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 (January/February 1978).
3. Official Methods of Analysis - 14th Edition, method 43.292-43.296, AOAC: Arlington, Virginia (1984).
4. Official Methods of Analysis - 1st Supplement, 14th Edition, Method 3.A01-3.A04, AOAC, Arlington, Virginia (1985).
5. U.S. Environmental Protection Agency Contract Laboratory program, Statement of Work, Inorganic Analysis, Multimedia, Multi-concentration, S.O.W. 7/88.
6. "Inductively Coupled Plasma-Atomic Emission Spectrometric Method of Trace Element Analysis of Water and Wastes," Method 200.7, edited by Theodore D. Martin and John F. Kopp, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
7. "Method Procedures, Analytical Chemistry Department, Inorganic Chemistry." Method MP-ICPS-MA, Hazleton Laboratories America, Inc., Madison, Wisconsin.

## Mercury by Cold Vapor Atomic Absorption

### SCOPE:

This method is applicable to most materials including animal tissues, plants, soils.

### PRINCIPLE:

Sample weight: 2.00 g  
Sample volume: 100 mL.

Samples are digested with a mixture of sulfuric and nitric acid. Mercury is reduced with sodium borohydride for determination. The amount of mercury is determined at a wavelength of 253.7 nm by comparing the signal of the unknown sample, measured by the atomic absorption spectrophotometer with the MHS-20 hydride generation unit, with the signal of the standard solutions.

Using a 2.0-g sample, the lowest detection limit of this assay is 0.025 ppm.

### REFERENCES:

1. Digestion: Analyst, 86:608 (1961) with modifications.
2. Determination: Analytical Chemistry, 40:2085 (1968).
3. Test Methods for Evaluating Solid Waste, EPA Publication No. SW-846, 2nd Ed., Methods 3030, 3040 or 3050 and 7470, U.S. EPA: Washington, D.C. (revised April 1984).

## Arsenic by Graphite Furnace

### SCOPE:

This method is applicable to animal tissues, plants, sediments, sludges, and soils.

### SAMPLE PREPARATION:

1) Animal or Plant Tissue

Digest 1.00 g with nitric acid in a microwave digester. Transfer to 100 mL.

2) Sediment or Soil

Digest 1.00 g with nitric acid and 30% hydrogen peroxide using covered glass beakers on hot plates. Transfer to 100 mL.

PRINCIPLE:

The amount of arsenic is determined at a wave length of 193.7 nm by comparing the signal of the unknown sample, measured by the graphite furnace atomic absorption spectrophotometer, with the signal of the standard solutions. The method of standard additions is used where interferences are indicated. Nickel matrix modification is employed in the analysis.

Using a 1.00-g sample, the lowest detection limit of this assay is 0.1 ppm.

REFERENCES:

1. Test Methods for Evaluating Solid Waste, EPA Publication No. SW-846, 2nd Ed., Methods 3030, 3040 or 3050 and 7060, U.S. EPA: Washington, D.C. (revised April 1984).
2. Contract Laboratory Program Statement of Work No. 785, Method 206.2 CLP-M, U.S. EPA: Cincinnati, Ohio.

Selenium by Graphite Furnace

SCOPE:

This method is applicable to animal tissues, plants, sediments, sludges, and soils.

SAMPLE PREPARATION:

1) Animal or Plant Tissue

Digest 1.00 g with nitric acid in a microwave digester. Transfer to 100 mL.

2) Sediment or Soil

Digest 1.00 g with nitric acid and 30% hydrogen peroxide using covered glass beakers on hot plates. Transfer to 100 mL.

PRINCIPLE:

The amount of selenium is determined at a wavelength of 196.0 nm by comparing the signal of the unknown sample, measured by the graphite furnace atomic absorption spectrophotometer, with the signal of the standard solutions. The method of standard additions is used along with nickel matrix modification in the analysis.

Using a 1.00-g sample, the lowest detection limit of this assay is 0.1 ppm.

REFERENCE;

1. Test Methods for Evaluating Solid Waste, EPA Publication No. SW-846, 2nd Ed., Methods 3030, 3040, or 3050 and 7740, U.S. EPA: Washington, D.C. (revised April 1984).

Acetylcholinesterase Assay with Reactivation

SCOPE:

This protocol for acetylcholinesterase activity includes tests for reactivation following organophosphate and carbamate exposure.

TISSUE PREPARATION:

Brain tissue was allowed to partially thaw at room temperature. Tissue was minced with a scalpel and an approximately 0.5 g subsample was diluted 1:10 w/v with pH 7.4 Tris buffer. Diluted samples were homogenized for 30 sec with a Polytron (Brinkman Instruments, Westburg NY) tissue grinder at high speed. A 0.5 ml aliquot of the homogenized sample was

diluted 1:10 with 7.4 Tris buffer (v/v - final concentration 1:200 for avian brains) and thoroughly mixed.

#### ANALYTICAL METHOD:

Each sample was run in triplicate. DTNB (5,5'-dithiobis-2-nitrobenzoic acid), pH 8.0 Tris buffer, the homogenate, and acetylthiocholine iodide as substrate were added one at a time to the labeled wells of a 96-well plate. Immediately upon the addition of acetylthiocholine iodide, the plate was inserted into a Spectra Max 250 (molecular Devices, Sunnyvale, CA) plate reader set at 28°C. Assays occurred over two minutes and involved 11 readings. At the end of the assay period, the mean and coefficient of variation (CV) was calculated for each set of three replicates. Analyses were deemed acceptable if CV<10%.

Reactivation for organophosphate exposure was conducted by adding 2 PAM to a vial containing the homogenate and incubating at 24°C for 45 minutes.

Carbamate reactivation was done by taking the diluted homogenate and incubating at 31°C for 1 hour.

#### REFERENCES:

Ellman, G.L., K.D. Courtney, V. Andres, and R.M. Featherstone. 1961. *Biochem. Pharm.* 7:88-95.

Martin, A., G. Norman, P.I. Stanley, and G.E. Westlake. 1981. *Bull. Environ. Contam. Toxicol.* 26:775-780.

Stanley, P.I. 1996. *Arch. Environ. Contam. Toxicol.* 25:315-321.