U.S. Department of the Interior—U.S. Geological Survey

SYNTHETIC ORGANIC COMPOUNDS AND CARP ENDOCRINOLOGY AND HISTOLOGY IN LAS VEGAS WASH AND LAS VEGAS AND CALLVILLE BAYS OF LAKE MEAD, NEVADA, 1992 AND 1995

Water-Resources Investigations Report 96-4266

Nevada Basin and Range Study Unit
National Water-Quality Assessment Program

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ABSTRACT

The Nevada Basin and Range study unit of the National Water-Quality Assessment Program, U.S. Geological Survey, in cooperation with the National Park Service, National Biological Service, and U.S. Fish and Wildlife Service, investigated the occurrence of organochlorines and semivolatile industrial compounds in the water column, bottom sediment, and carp (*Cyprinus carpio*) tissue at five sites in Las Vegas Wash and Lake Mead. Endocrine systems of carp were assessed by analyzing concentrations of female and male sex-steroid hormones, 17β-estradiol and 11-ketotestosterone, and vitellogenin (an estrogen-controlled egg protein) in blood-plasma samples. The histology of carp gonads, hepatopancreas, kidney, gill, and lower intestine were analyzed for effects that can result from endocrine disruption or exposure to toxicants.

Organochlorines (pesticides and industrial compounds) and semivolatile industrial compounds were detected in semipermeable membrane devices and bottom-sediment samples; only organochlorines were detected in carp-tissue samples. Concentrations of organochlorines were higher in Las Vegas Wash and Bay than in Callville Bay (the reference site) for the three media that were sampled. Results of a carp-tissue bioassay indicated the presence of dioxins or furans with low toxic-equivalent factors relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin in Las Vegas Wash and Las Vegas and Callville Bays. Patterns of necrosis observed in hepatopancreas and kidney samples from carp are consistent with long-term subchronic exposure to toxicants. Polycyclic aromatic hydrocarbons, phthalates, and phenols also were detected at higher concentrations in bottom-sediment samples from Las Vegas Bay than in a comparable sample from Callville Bay. Polycyclic aromatic hydrocarbons were detected in samples from semipermeable membrane devices from all sites.

Endocrine disruption in carp from Las Vegas Wash and Bay, as compared to Callville Bay, is evidenced by high concentrations of 11-ketotestosterone levels in blood-plasma samples of female carp in Las Vegas Wash, low concentrations in male carp from Las Vegas Bay, and low 17β-estradiol concentrations in male carp from Las Vegas Bay. The most compelling evidence of endocrine disruption is the presence of vitellogenin in blood-plasma samples of male carp from Las Vegas Wash and Bay and elevated concentrations in female carp from Las Vegas Bay.

Many of the organochlorines and semivolatile industrial compounds detected in semipermeable membrane devices, bottom sediment, and carp tissue from Las Vegas Wash and Bay have been linked to endocrine disruption in fish by previous investigations of other areas. The endocrine disruption observed in carp from Las Vegas Wash and Bay could be due to the presence of these compounds.

Aerial view of lower Las Vegas Wash and Las Vegas Bay of Lake Mead. View to the northwest, Oct. 12, 1995. Photograph by A.S. VanDenburgh.

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INTRODUCTION

The Nevada Basin and Range (NVBR) study unit of the U.S. Geological Survey's National Water-Quality Assessment (NAWQA) Program is investigating the status of, trends in, and factors affecting water quality in the Las Vegas area (fig. 1). A principal objective of the investigation is to assess the effects of urbanization on the quality of area water resources.

Las Vegas Wash (fig. 1) is the surface-water outlet for the Las Vegas area. The wash transports stormwater runoff, shallow ground-water discharge, and tertiary-treated sewage effluent from the Las Vegas area to Las Vegas Bay of Lake Mead on the Colorado River (fig. 1). Most of the flow—about 96 percent in 1993—transported in the wash to Las Vegas Bay is treated effluent from city and county sewage-treatment facilities. Storm-water runoff, shallow ground-water discharge, and treated sewage effluent from Las Vegas and other urbanized areas in the valley have the potential to affect the water quality of Las Vegas Wash and Lake Mead.

In 1992, NVBR personnel collected bottom-sediment samples from Las Vegas Wash (site 1, fig. 1 and table 1) upstream from the discharge of treated sewage effluent and Las Vegas Bay (site 3) during a survey to determine if synthetic organic compounds (organochlorines and semivolatile industrial compounds) were present. Organochlorines (pesticides

Base from U.S. Geological Survey digital data, 1:100,000, 1978-87. Albers Equal-Area Conic projection. Standard parallels 29°30' and 45°30', central meridian-115°00'. Land use from Land Use and Land Cover, OFR Land-Use Series, 1973-77

EXPLANATION

Urban area

▼2 **Data-collection site and number**

Location of study area

Figure 1. Data-collection sites in Las Vegas area, Nevada.

and industrial compounds), polycyclic aromatic hydrocarbons (PAH's), phthalates, and phenols were detected at one or both of these sites. Many of these compounds are persistent in the environment, are relatively insoluble in water, and strongly partition into sediment organic material and lipid tissues of organisms (Smith and others, 1988).

Compounds referred to as organochlorines include chlorinated pesticides and their metabolites. polychlorinated biphenyls (PCB's), dioxins, and furans. PCB's have been used as plasticizers and hydraulic lubricants, in heat-transfer systems, and as dielectric fluids in electrical capacitors and transformers (Smith and others, 1988). Dioxins and furans were produced inadvertently during the manufacture of herbicides and PCB's. and are formed during municipal waste combustion (Sijm and Opperhuizen. 1996); they are commonly discharged to surface waters in effluents from chemical manufacturing plants, leather tanneries, kraft-pulp mills, wood-processing plants, and sewage-treatment plants (Smith and others, 1988).

PAH's originate from natural and human sources. They are produced mainly by high-temperature pyrolytic reactions such as municipal incineration or forest fires, but a few are produced commercially for use in mothballs, pesticides, fungicides, dyes, wetting agents. synthetic resins, cutting fluids, solvents, and lubricants (Smith and others, 1988). Phthalates are used extensively as plasticizers to manufacture products from polymers of vinyl chloride, propylene, ethylene, and styrene (Smith and others, 1988).

Phenols are used in the production of phenolic resins, germicides, herbicides, fungicides, pharmaceuticals, dyes, plastics, and explosives (Smith and others, 1988). Most phenols are not persistent and are highly soluble; however, highly chlorinated phenols are persistent and tend to partition into sediment organic matter and lipids of aquatic organisms (Smith and others, 1988).

Interest in environmental contaminants that may affect endocrine systems of animals by causing hormone imbalance, commonly referred to as endocrine disruption, has grown during the past 40 years. A symposium on estrogen in the environment (McLachlan, 1980) increased the debate about effects of environmental contaminants on endocrine systems. More recently, Colborn and Clement (1992) concluded that many synthetic organic compounds have the potential to disrupt the endocrine systems of animals, including humans; these compounds include organochlorines, PAH's, phthalates, and phenols (Thomas, 1988; Colborn and others, 1993).

Recent studies have found evidence of endocrine disruption in fish from contaminated ecosystems. Fitzsimons (1990) reported lower sex-steroid hormone levels in male lake trout (*Salvelinus namaycush*) of Lake Ontario compared with fish from less-polluted sites. Additionally, an inverse relation was found between 11-ketotestosterone and contaminant body burdens in trout from three of the Great Lakes. Male and female white suckers (*Catostomus commersoni*) and lake whitefish (*Coregonus clupeaformis*) in the vicinity of pulp-mill effluent were found to have depressed gonadal sex-steroid hormones, delayed maturity, lowered reproductive capability. and reduced secondary sex characteristics (Munkittrick and others, 1992).
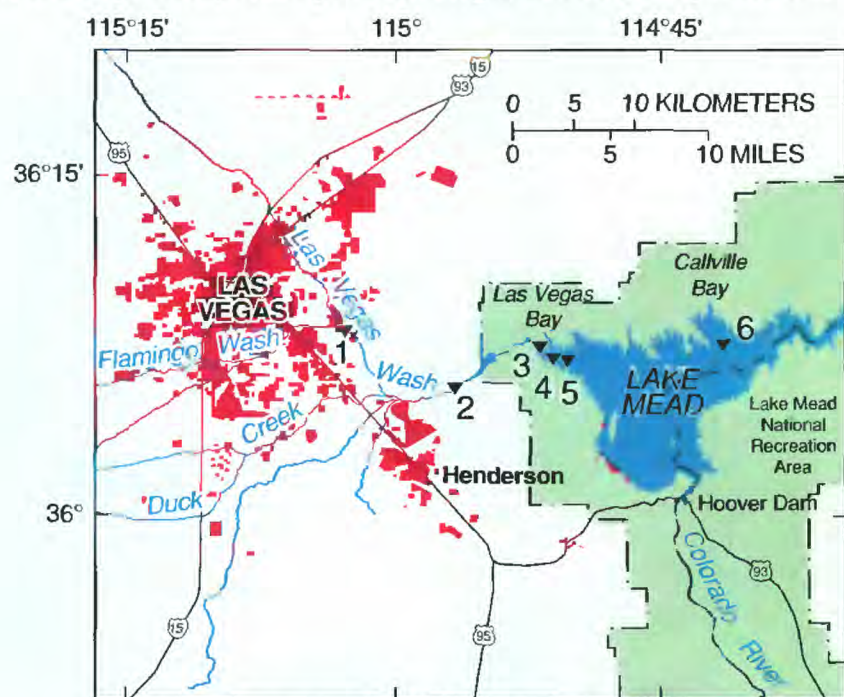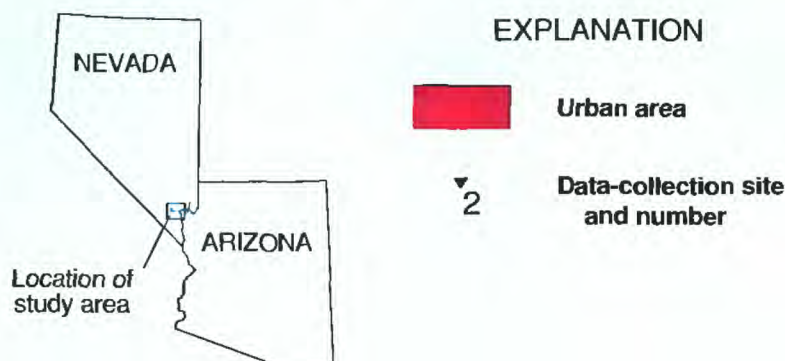
Other studies have measured vitellogenin to assess endocrine disruption. Vitellogenin is an estrogen-induced or dependent egg protein used in the production of egg yolk and normally synthesized by the livers of female egg-laying vertebrates (Specker and Sullivan, 1994). Male fish have the vitellogenin gene but it is normally suppressed; however, the gene can be activated by exposure to exogenous contaminants or xenoestrogens (LeGuellec and others, 1988). Several recent field studies have documented vitellogenin synthesis in carp males from streams affected by sewage effluent (Purdom and others, 1994; Folmar and others, in press).

Histologic abnormalities in gonads of fish with endocrine disruption can include multinuclear eggs or the presence of dark, bar-shaped structures in tubules of the testes (McLachlan and Arnold, 1996). Necrosis in tissues of organs can be caused by exposure to a toxicant or a combination of toxicants (J.S. Foott, U.S. Fish and Wildlife Service, written commun., 1995).

The potentially harmful effects that organochlorines and semivolatile industrial compounds can have on humans and aquatic wildlife prompted the National Park Service (NPS), which administers the Lake Mead National Recreation Area, and the NVBR to begin a cooperative investigation to determine the occurrence and distribution of these compounds in Las Vegas Wash and Lake Mead. Concurrent assessments of the endocrine systems and histology of selected organs in carp (*Cyprinus carpio*) were made in cooperation with the National Biological Service (NBS), which on October 1, 1996, became part of the U.S. Geological Survey (USGS), and the U.S. Fish and Wildlife Service (USFWS). Although the investigation is currently (1996) ongoing, data were collected in 1995 for Las Vegas Wash (site 2 on fig. 1 and table 1), Las Vegas Bay (sites 3, 4, and 5), and Callville Bay (site 6). Callville Bay, which is in a part of Lake Mead upstream from Las Vegas Bay, was sampled as a reference site for comparisons with Las Vegas Wash and Bay.

This report presents preliminary results of the 1995 investigation and includes the 1992 sample information.

ACKNOWLEDGMENTS

Table 1. Data-collection sites and activities in Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, 1992 and 1995

[Abbreviation: SPMD, semipermeable membrane device.]

Site no. (fig. 1)	Site name	Depth of SPMD and bottom-sediment samples (feet below water surface)	SPMD sample Organochlorines and industrial compounds	SPMD sample Dioxins and furans	Bottom-sediment sample Organochlorines and industrial compounds	Bottom-sediment sample Dioxins and furans	Common carp
1	Las Vegas Wash below Flamingo Wash				[1]X		
2	Las Vegas Wash near Henderson		X	X			X
3	Las Vegas Bay near Las Vegas Wash inlet	[2]10	[2]X				X
		[1]18			[1]X	[1]X	
		23	X	X			
		24			X	X	
4	Las Vegas Bay C Buoy	20	X				
		55	X				
		70	X	X			
		77			X	X	
5	Las Vegas Bay B Buoy	20	X				
		70	X				
		151			X	X	
6	Callville Bay A Buoy	15	X				X
		70	X	X			
		171			X	X	

[1] Sampled in September 1992; all other samples are from 1995.

[2] Duplicate sample set collected for quality-assurance purposes.

Collecting bottom-sediment sample near mouth of Callville Bay (site 6), July 12, 1995. Photograph by Kenneth J. Covay.

INVESTIGATION METHODS

The occurrence of organochlorines and semivolatile industrial compounds in the aquatic environment is important for human-health and wildlife issues. Detection of these compounds in water is problematic because of the transiency of many point-source discharges and the low concentrations of these relatively insoluble compounds, which result from non-point sources (Ellis and others, 1995). Therefore, semipermeable membrane devices (SPMD's) were used to sample for these compounds in the water column.

SPMD's are passive sampling devices that contain fish lipid or triolein in a low-density polyethylene tube (Huckins and others, 1990). They are effective in sequestering dissolved organochlorines and semivolatile industrial compounds from water and in assessing their bioavailability (Huckins and others, 1990; Huckins and others, 1993; Ellis and others, 1995). Compounds are recovered from the SPMD's by dialysis using an organic solvent, cleaned up by gel permeation chromatography, and analyzed by gas chromatography-mass spectrometry. SPMD's used in this study were manufactured by Environmental Sampling Technologies (St. Joseph, Mo.), which also provided dialysis and clean-up services.

The SPMD's in Las Vegas Wash were attached to a metal fence post in the wash channel. The SPMD's at lake sites were attached to buoy anchor cables at depths (table 1) selected to obtain samples from above and below the thermocline, which was determined by measurements of temperature profiles. SPMD's were installed for 5 weeks, from early June to mid-July 1995. For quality-assurance purposes, a duplicate set of SPMD's was installed at site 3 (table 1) and a set of trip blanks (SPMD's transported to the sampling site) was exposed to the atmosphere during the installation and retrieval of sample SPMD's.

Analyses of organochlorines and semivolatile industrial compounds in SPMD's were made by the USGS National Water Quality Laboratory (Arvada, Colo.). Analyses of dioxins and furans were made by Quanterra Environmental Services (Sacramento, Calif.). Method blanks were analyzed as part of laboratory quality-assurance procedures. Although models have been developed to estimate water concentrations from SPMD concentrations (Huckins and others, 1993; Ellis and others, 1995), SPMD concentrations are used herein only to determine the presence of these compounds and compare the types and relative concentrations of compounds detected at sampling sites.

Bottom-sediment samples were collected and processed according to NAWQA protocols (Shelton and Capel, 1994). Samples were analyzed for dry-weight concentrations of organochlorines and semivolatile industrial compounds by the USGS National Water Quality Laboratory, and for dry-weight concentrations of dioxins and furans by Quanterra Environmental Services.

Carp were collected from Las Vegas Wash by seining and from Las Vegas and Callville Bays by boat electrofishing. Blood samples were collected with a sterile syringe and centrifuged; the plasma was removed, frozen with dry ice, and submitted to the Biotechnology for Evolutionary, Ecological, and Conservation Sciences Program Laboratory, University of Florida, Gainesville, for analyses of female and male sex-steroid hormones [17β-estradiol (E_2) and 11-ketotestosterone (11-KT)] and an egg protein (vitellogenin).

Blood-plasma samples were analyzed for E_2 and 11-KT using radioimmunoassay (RIA) procedures. Duplicate samples were extracted with diethyl ether, analyzed for E_2 and 11-KT, and corrected for extraction efficiency. Standard curves were prepared using buffers with known amounts of radioinert E_2 or 11-KT. Cross-reactivities of the E_2 antiserum with other female sex steroids were 11.2 percent for estrone, 1.7 percent for estriol, less than 1.0 percent for 17α-estradiol and androstenedione, and less than 0.1 percent for all other steroids examined.

National Park Service divers retrieve a semipermeable membrane device from buoy in Las Vegas Bay (site 4), July 12, 1995. Photograph by Kenneth J. Covay.

Cross-reactivities of the 11-KT antiserum with other male sex steroids were 9.65 percent for testosterone, 3.7 percent for α-dihydrotestosterone, less than 1.0 percent for androstenedione, and less than 0.1 percent for all other steroids examined. A pooled sample was assayed serially to develop inhibition curves, which were determined to be equivalent to the respective standard curves. The standard curves were then used to determine E_2 and 11-KT concentrations.

Vitellogenin in blood-plasma samples of carp was assayed and quantified by using an enzyme-linked immunosorbent assay (ELISA) protocol, as described by Folmar and others (in press), and the Bradford assay (Peterson, 1983). For this investigation, the detection limit for vitellogenin is about 1 mg/mL. Carp vitellogenin was purified by chromatography and used to develop a standard curve. Duplicate samples were analyzed. Nonspecific background-binding values were determined by using male blood plasma from reference animals at concentrations equivalent to those of study samples. The background values were subtracted from the sample values. In addition, a reference sample with a known concentration of vitellogenin was used to test for inter- and intra-assay variation. The standard curve was used to determine the vitellogenin concentration for each study sample, which was then corrected for dilution.

Organ samples (gonad, hepatopancreas[1], kidney, gill, and lower intestine) were collected from the same carp that yielded the blood samples. Gonad samples (portions of testes and ovaries) were fixed in a solution of 71 percent saturated aqueous picric acid, 24 percent formalin, and 5 percent glacial acetic acid. Gonad samples were submitted to the University of Florida, Gainesville, for histologic analysis to determine if the carp samples were at equivalent sexual maturation levels and if any abnormalities were associated with endocrine disruption.

Female and male gonads were classified according to the level of sexual development. In female carp, the size and

Collecting blood from carp. Photograph by Kenneth J. Covay.

Semipermeable membrane device is removed from protective shield after retrieval. Photograph by Kenneth J. Covay.

[1] In carp, the hepatopancreas has both liver and pancreas functions.

number of ova and vitelline granules, and granulomatous inflammation were considered. In male carp, spermatogenic activity in the germinal epithelium, thickness of the germinal epithelium, and proliferation and maturation of spermatozoa were considered.

Hepatopancreas, kidney, gill, and lower intestine samples were fixed in a solution of 43 percent ethyl alcohol (95 percent), 29 percent formalin, 14 percent glacial acetic acid, and 14 percent distilled water. Samples were submitted to the USFWS California-Nevada Fish Health Center (Anderson, Calif.) for histologic analysis.

The carp carcasses were then wrapped in hexane-rinsed aluminum foil and frozen to -20°C. These samples were submitted to the USEPA Region 9 Laboratory (Richmond, Calif.) for analysis of organochlorines and semivolatile industrial compounds. A 2-in-wide, mid-body, vertical cross-section was removed from each carp and homogenized. Aliquots of this homogenate were extracted and analyzed for wet-weight concentrations of organochlorines according to protocols of the Superfund Contract Laboratory Program Organic Statement of Work (revision OLM03.1), which is equivalent to USEPA method 8080 (Rich Bauer, USEPA, oral and written communs., 1996). Aliquots also were analyzed for 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) toxic equivalents by the NBS Midwest Science Center (Columbia, Mo.), by using a modification (Tillitt and Alt, 1996) of the PLHC-1 bioassay procedure (Tillitt and others, 1991) and a slope-ratio assay described by Ankley and others (1991).

Statistical methods used in this report include graphical presentations showing data distributions and nonparametric tests to evaluate general and specific differences among groups of data. A chi-squared approximation of the Kruskal-Wallis test (Hollander and Wolfe, 1973) was applied to ranked data to evaluate general differences among groups. For evaluating specific differences among more than two groups, the Duncan multiple-range test (Steel and Torrie, 1960) was applied to ranked data.

OCCURRENCE OF SYNTHETIC ORGANIC COMPOUNDS

Water

SPMD's were deployed to sample the water column in Las Vegas Wash (site 2 on fig. 1 and table 1), Las Vegas Bay (sites 3, 4, and 5), and Callville Bay (site 6). The principal compounds detected were organochlorines, polycyclic aromatic hydrocarbons, and phthalates. The number and combined concentration of organochlorines were highest in Las Vegas Wash, generally decreased in Las Vegas Bay, and were much lower in Callville Bay (fig. 2). Organochlorines detected at every site in Las Vegas Wash and Bay include hexachlorobenzene; DCPA (dacthal); trans-chlordane; cis-chlordane; trans-nonachlor; dieldrin; p,p'-DDE; p,p'-DDD; and total polychlorinated biphenyls (PCB's). Concentrations of these compounds had areal distributions that were similar to the distribution of total organochlorines (except dacthal, which was detected at the highest concentrations in Callville Bay).
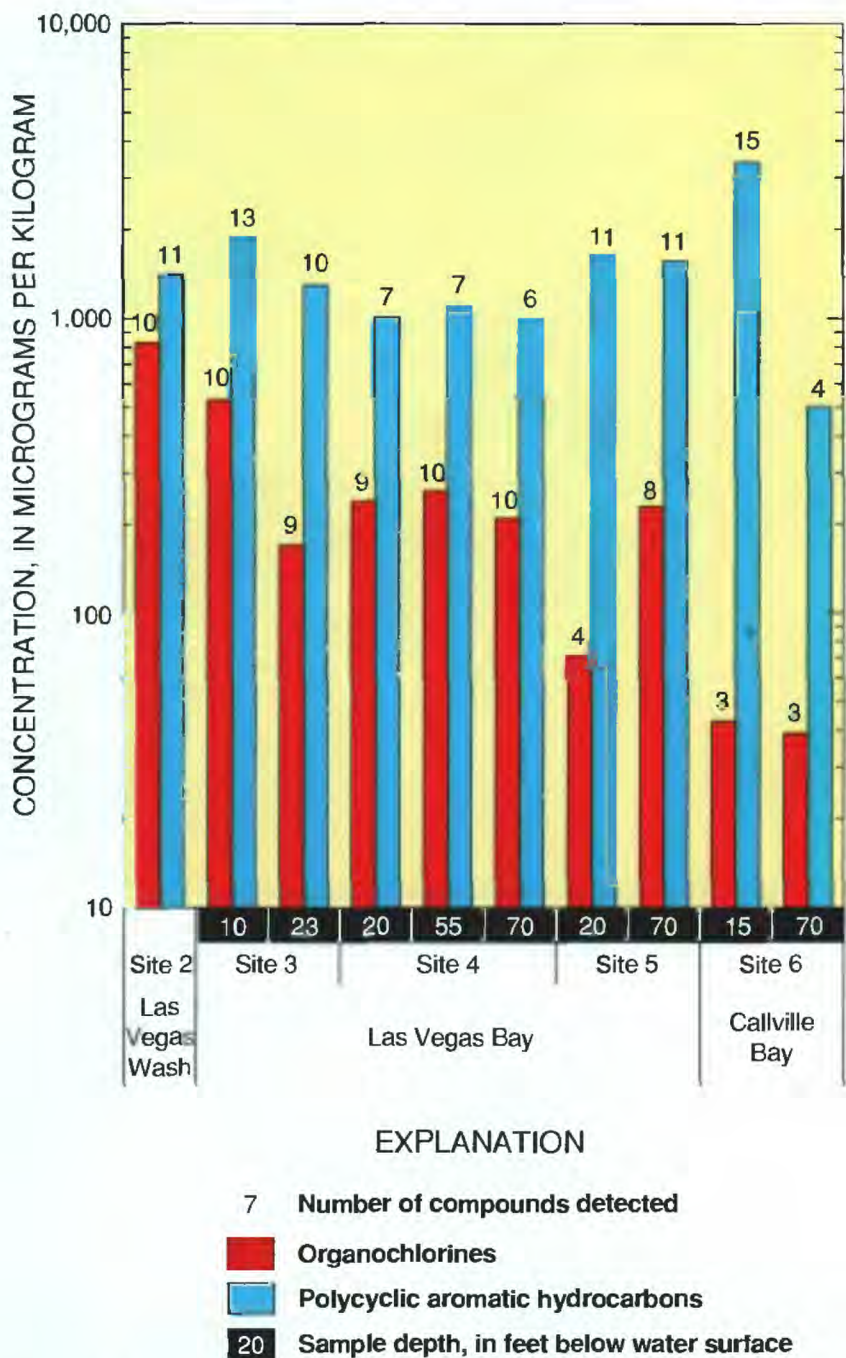

Figure 2. Numbers and combined concentrations of organo-chlorines and polycyclic aromatic hydrocarbons detected in samples from semipermeable membrane devices, Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, June-July 1995.
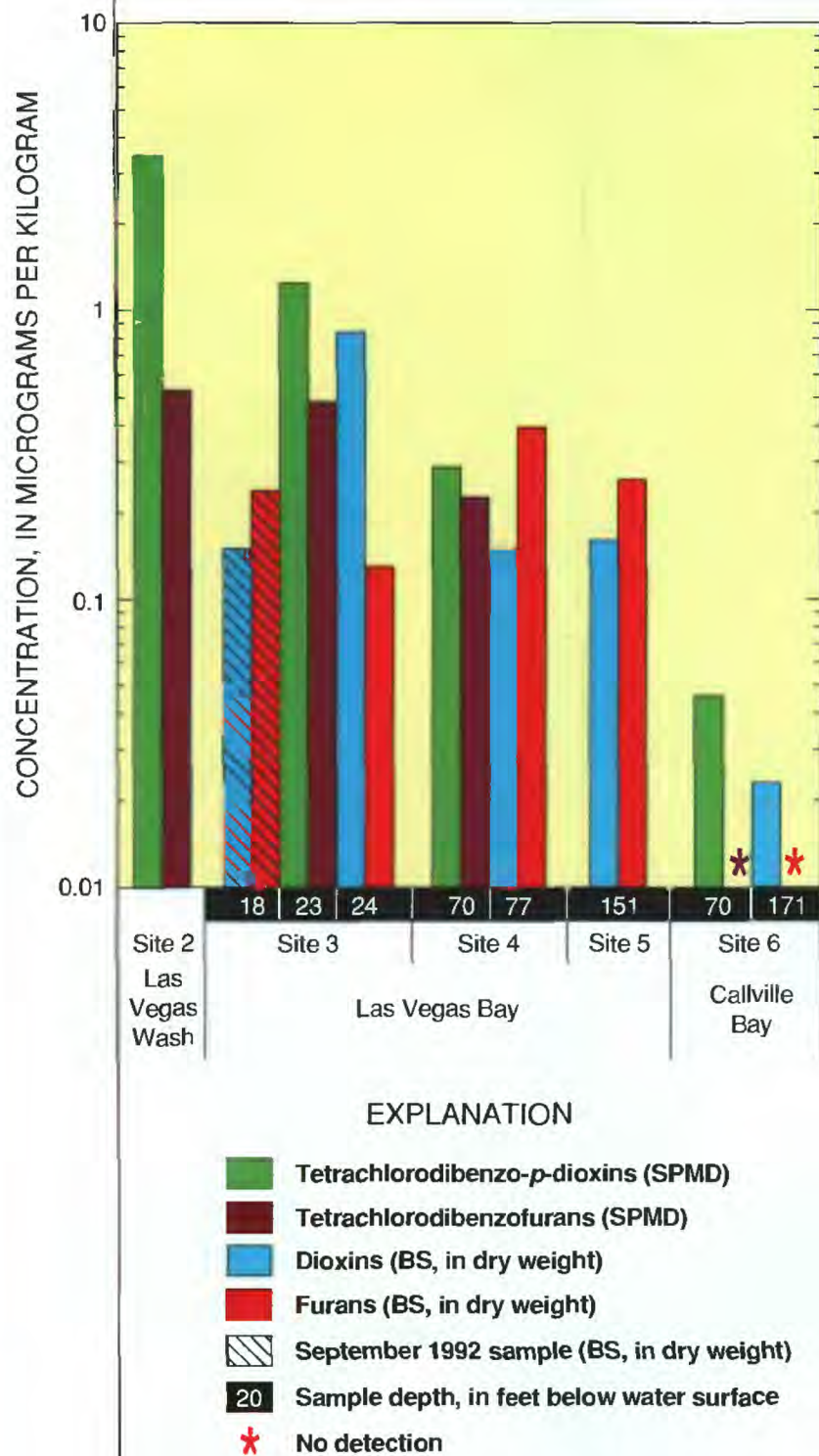

Figure 3. Combined concentrations of dioxins and furans detected in samples from semipermeable membrane devices (SPMD) and bottom sediment (BS), Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, September 1992 and June-July 1995.

These areal distributions indicate that Las Vegas Wash is a source of these compounds for Las Vegas Bay.

Combined concentrations of tetrachlorodibenzo-p-dioxins (TCDD's) in SPMD's were highest in Las Vegas Wash and de-creased in Las Vegas Bay as the distance from the wash inlet increased (fig. 3). These compounds also were detected in Callville Bay, but at concentrations that were nearly two orders of magnitude lower than in Las Vegas Wash. The compound 2,3,7,8-TCDD is the most toxic synthetic organic compound studied under laboratory conditions (Eisler, 1986); other dioxin and furan compounds are assigned toxic equivalent factors (TEF's) on the basis of their relative toxicity in comparison to 2,3,7,8-TCDD (U.S. Environmental Protection Agency, 1989). Combined concentrations of tetrachlorodibenzofurans (TCDF's) also were highest in Las Vegas Wash and decreased in the Las Vegas Bay as distance from the wash inlet increased. The most toxic dioxin or furan detected was 2,3,7,8-TCDF

(TEF= 0.1) in Las Vegas Wash (site 2) and Las Vegas Bay (sites 3 and 4). SPMD's for dioxin and furan analyses were not deployed at site 5 in Las Vegas Bay. The areal distributions of dioxins and furans indicate that Las Vegas Wash is a source of these compounds for Las Vegas Bay.

The number and combined concentrations of PAH's in SPMD's (fig. 2) in outer reaches of Las Vegas Bay (site 5) and Callville Bay (site 6) were similar to or higher than those in Las Vegas Wash (site 2). PAH's detected at all sites in Las Vegas Wash and Bay include 2,3,6-trimethylnaphthalene; 4,5-methylenephenanthrene; phenanthrene; fluoranthene; pyrene; and chrysene. Concentrations of these compounds had areal distributions that were similar to the areal distribution of total PAH's (except pyrene, which was highest in Las Vegas Wash, decreased in Las Vegas Bay as distance from the wash inlet increased, and was lowest in Callville Bay). These areal distributions indicate that Las Vegas Wash may be a source of some PAH's for Las Vegas Bay, but also that there are other, perhaps more general, sources of PAH's to Lake Mead.

Large numbers and concentrations of phthalates were detected in all the SPMD samples, including the trip blank and the method blank. Because phthalates are used as plasticizers for polyethylene, the polymer from which the SPMD tubes are made, these sampling devices probably were the source of most detected phthalates.

Bottom Sediments

Bottom-sediment samples were collected from Las Vegas Wash (site 1) and Las Vegas Bay (site 3) in 1992 and from Las Vegas Bay (sites 3, 4, and 5) and Callville Bay (site 6) in 1995. Compounds detected in the sample from Las Vegas Wash were PAH's and phthalates (fig. 4). Compounds detected in samples from Las Vegas Bay included organochlorines, PAH's, phthalates, and phenols (fig. 4). Site 1 on Las Vegas Wash is upstream from city and county sewage-treatment-plant discharges and industrial activities in the vicinity of Henderson, Nev.; the absence of organochlorines and phenols in this sample may be because it is upstream from these potential sources.

Organochlorines detected in bottom sediment from the three Las Vegas Bay sites sampled in 1995 included p,p'-DDD and p,p'-DDE, which were not detected in the sample from Callville Bay. Combined concentrations of dioxins and furans detected in samples from Las Vegas Bay were an order of magnitude higher than the sample from Callville Bay (fig. 3). Organochlorines, dioxins, and furans were not analyzed in the 1992 sample from Las Vegas Wash (site 1). The furan 2,3,7,8-TCDF (TEF=0.1) was detected in samples from all three Las Vegas Bay sites. Other dioxins or furans detected in samples from all three sites were octochlorodibenzo-p-dioxin (OCDD; TEF=0.001) and octochlorodibenzofuran (OCDF; TEF=0.001). The furan 1,2,3,7,8-pentachlorodibenzofuran (PeCDF; TEF=0.05) was detected in samples from sites 4 and 5.

Concentrations of combined PAH's and combined phthalates were about an order of magnitude greater in bottom-sediment samples from Las Vegas Wash and Bay than in the sample from Callville Bay (fig. 4). The only PAH detected at all Las Vegas Wash and Bay sites was 2,6-dimethylnaphthalene;

pyrene was detected at all sites except site 5. The only phthalate detected at all Las Vegas Wash and Bay sites was bis(2-ethylhexyl)phthalate. However, di-n-butylphthalate and diethylphthalate were detected at all three Las Vegas Bay sites sampled in 1995. The phenolic compound p-cresol was detected in high concentrations at site 3 in Las Vegas Bay during 1992 and 1995 (4,050 and 930 µg/kg, dry weight, respectively), but was not detected in Callville Bay during 1995.
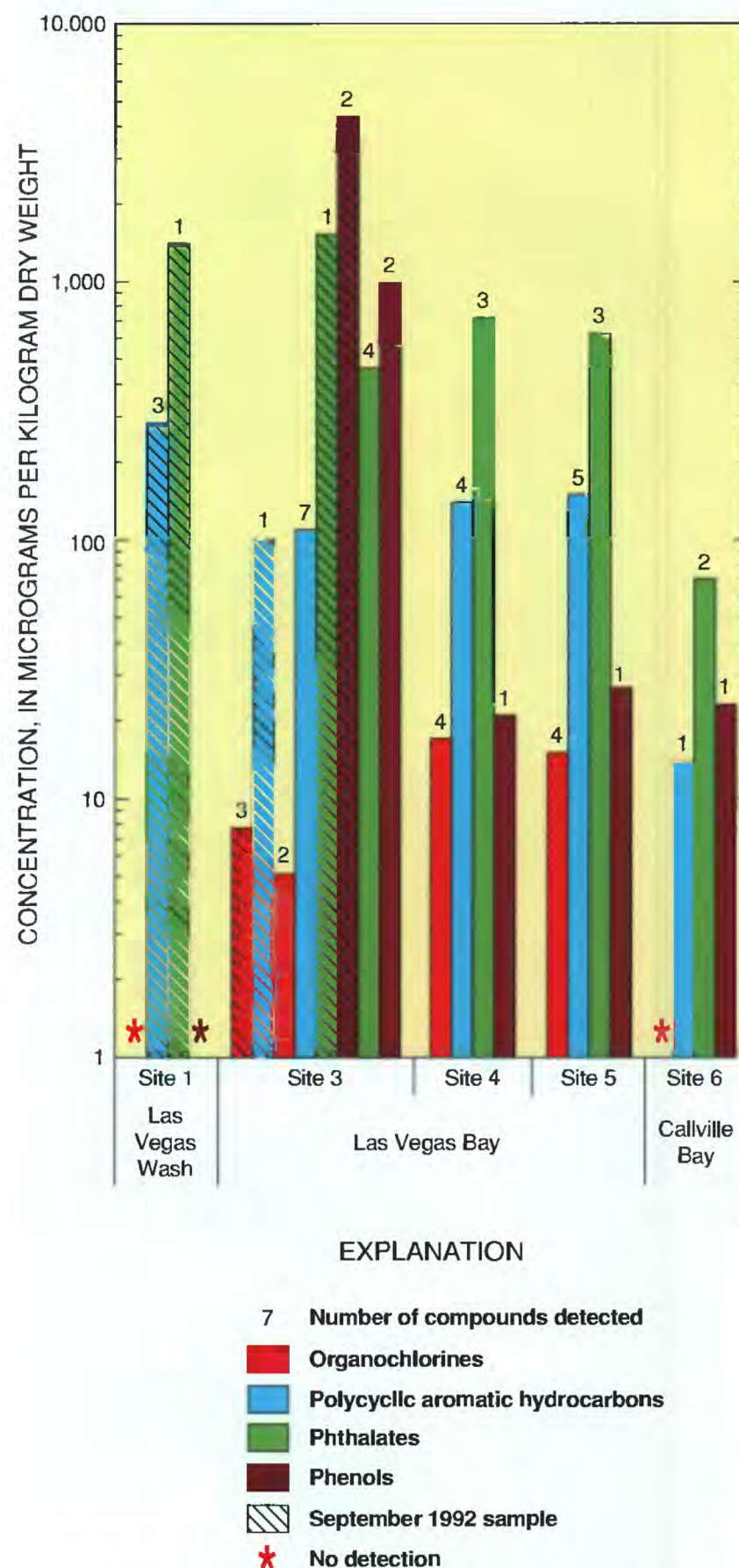

EXPLANATION

7	**Number of compounds detected**
▉ (red)	**Organochlorines**
▉ (blue)	**Polycyclic aromatic hydrocarbons**
▉ (green)	**Phthalates**
▉ (dark red)	**Phenols**
▧	**September 1992 sample**
✱	**No detection**

Figure 4. Numbers and combined concentrations of organochlorines, polycyclic aromatic hydrocarbons, phthalates, and phenols detected in bottom-sediment samples, Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, September 1992 and July 1995.

Fish Tissue

Twenty organochlorines were detected in carp-tissue samples. Samples from Las Vegas Wash contained more organochlorines (18) than samples from Las Vegas Bay (17) and Callville Bay (9). Samples from Las Vegas Bay had the highest concentrations of 13 organochlorines and samples from Las Vegas Wash had the highest concentrations of 5 organochlorines; samples from Callville Bay did not have the highest concentrations of any organochlorine detected. Samples from Las Vegas Wash and Bay had significantly higher concentrations of combined organochlorines (significance level p less than 0.05) than samples from Callville Bay (fig. 5)[1].

DDT residues (the sum of p,p'-DDT and its metabolites p,p'-DDE and p,p'-DDD) in carp-tissue samples were significantly higher (p less than 0.05) in Las Vegas Wash and Bay than in Callville Bay (fig. 5). The metabolite p,p'-DDE was detected in all samples, p,p'-DDD was detected in all samples from Las Vegas Wash and Bay, but in only one sample from Callville Bay, and p,p'-DDT was detected at all sites. In a sample from Las Vegas Bay, p,p'-DDE was detected at 230 micrograms per kilogram (μg/kg), wet weight. This sample also had the highest concentration of DDT residues (320 μg/kg, wet weight).

Median concentrations of DDT residues detected in cross-sectional carp-tissue samples from Las Vegas Wash (72 μg/kg, wet weight) and Las Vegas Bay (120 μg/kg, wet weight) exceeded the risk-based consumption limit of 60 μg/kg, wet weight of edible tissue, for the general population, assuming the consumption of more than 2 and 0 (zero) ounces of fish per month, respectively, and a protection level against a 1×10^{-6} increase in cancer risk (U.S. Environmental Protection Agency, 1994, table 3-17). The median concentration in samples from Las Vegas Wash also exceeded consumption-limit guidelines for chronic-systemic health effects in children, assuming consumption of more than nine, 4-ounce meals per 10-day period (U.S. Environmental Protection Agency, 1994, table 3-18). The median concentration detected in samples from Las Vegas Bay also exceeded consumption-limit guidelines for

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[1]Figures 5-8 show data distributions for concentrations of selected organic compounds. If the number of samples is less than 10, only the individual data points are plotted; if the number of samples is 10 to 14, a rectangular box showing the 25th, 50th (median), and 75th percentile values is plotted and outlying data points are shown; if the number of samples is 15 or more, whiskers extending to the 10th and 90th percentiles are added to the rectangular box and outlying data points are shown. This type of illustration is known as a boxplot. The graphical components are as follows.

15 ——— **Number of samples**

———— **Single data point**

90th percentile

75th percentile

50th percentile (median)

25th percentile

10th percentile
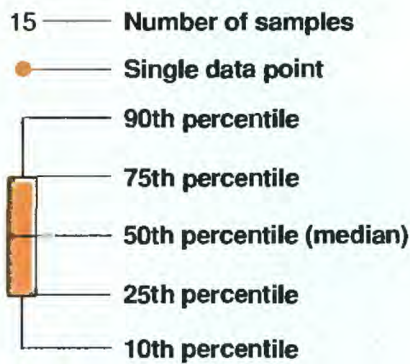
chronic-systemic health effects in the general population, assuming the consumption of more than 10, 12-ounce meals per 10-day period, and in children, assuming the consumption of more than six, 4-ounce meals per 10-day period (U.S. Environmental Protection Agency, 1994, tables 3-15, 3-18). The median concentration of DDT residues detected in samples from Callville Bay (9.5 μg/kg, wet weight) did not exceed any risk-based consumption limits. Although cross-sectional carp-tissue samples analyzed for this study are not equivalent to edible tissues of game fish, the results indicate that concentrations in edible portions of game fish may exceed some USEPA consumption limits.

Combined concentrations of Aroclors (sum of compounds found in Aroclors) in carp-tissue samples were significantly higher (p less than 0.05) in Las Vegas Wash and Bay than in Callville Bay (fig. 5). Aroclors are commercial mixtures of polychlorinated biphenyls and terphenyls (Smith and others,
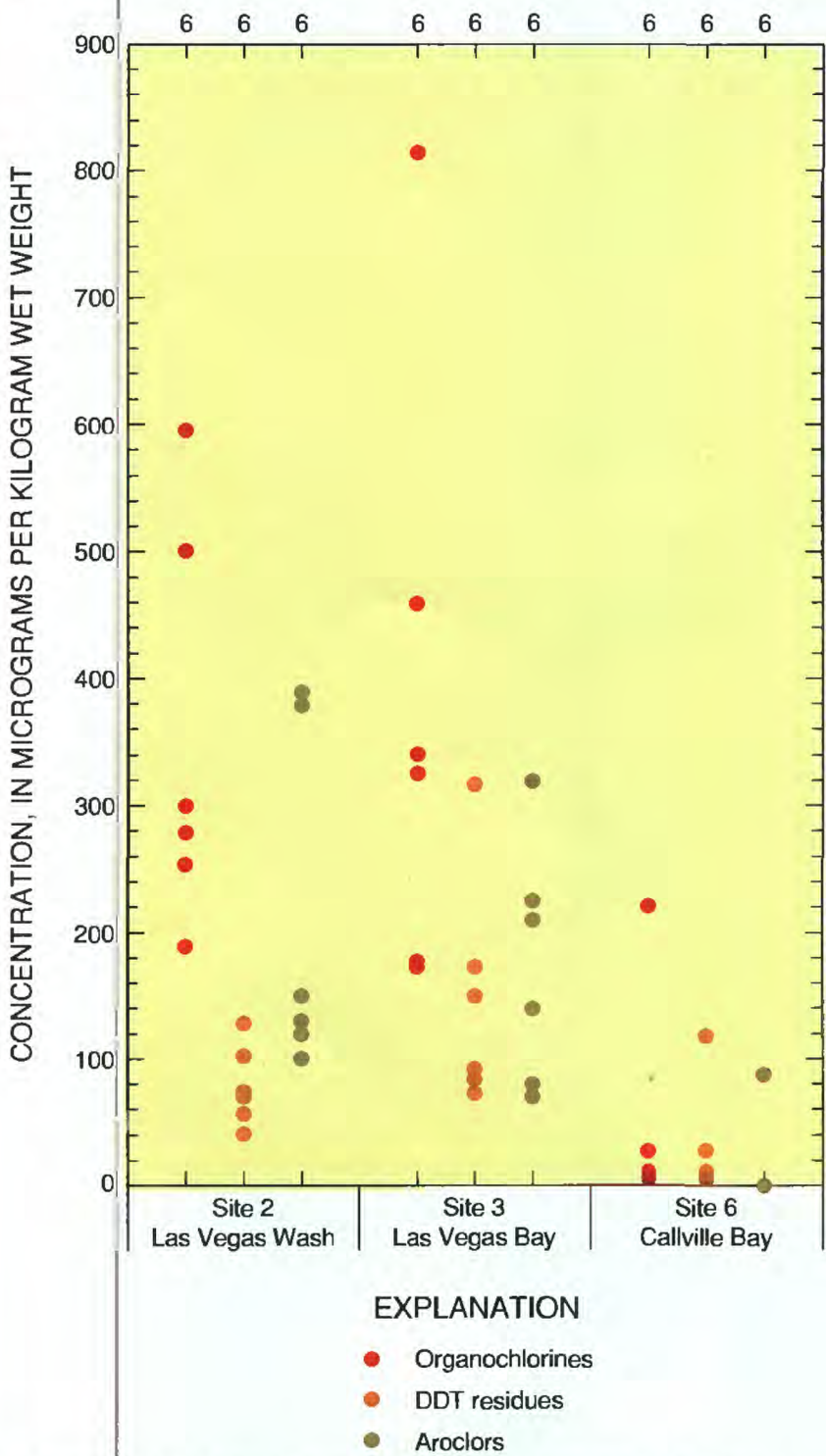

Figure 5. Combined concentrations of organochlorines, DDT residues (sum of p,p'-DDT, p,p'-DDD, and p,p'-DDE), and Aroclors (sum of compounds resembling Aroclors) detected in tissue samples from carp, Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, May 1995.

1988). A mixture of compounds resembling Aroclor 1254 was detected in 11 of 12 samples from Las Vegas Wash and Bay; the highest concentration of this mixture of compounds detected was 320 µg/kg, wet weight, in a sample from Las Vegas Bay—the same sample that had the highest combined concentration of DDT residues. The sample with the highest combined concentration of the mixture of compounds found in Aroclors (390 µg/kg, wet weight) was from Las Vegas Wash.

The median concentrations of the mixture of compounds resembling Aroclor 1254 detected in cross-sectional carp-tissue samples from Las Vegas Wash (130 µg/kg, wet weight) and Las Vegas Bay (140 µg/kg, wet weight) exceeded risk-based consumption limits for chronic-systemic health effects for the general population, assuming the consumption of more than one, 4-ounce meal per 10-day period (U.S. Environmental Protection Agency, 1994, table 3-68). These median concentrations also exceeded the risk-based consumption limit for chronic-systemic health effects for children, assuming any consumption per 10-day period (U.S. Environmental Protection Agency, 1994, table 3-71). These results indicate that concentrations in edible portions of game fish may exceed some USEPA consumption limits.

Results of the 2,3,7,8-TCDD toxic-equivalent bioassay analysis of carp-tissue samples indicate the presence of low levels of toxic equivalents of dioxins or furans in samples from Las Vegas Wash and Las Vegas and Callville Bays (Tillitt and Alt, 1996). These low levels of toxic equivalents could be due to low levels of highly toxic 2,3,7,8-TCDD, which was not detected in SPMD or bottom-sediment samples, or to the presence of less toxic dioxins and furans, which is consistent with SPMD and bottom-sediment results.

ASSESSMENT OF CARP ENDOCRINOLOGY AND HISTOLOGY

Concentrations of 11-ketotestosterone (11-KT) in blood-plasma samples from female and male carp collected from Las Vegas Wash (site 2), Las Vegas Bay (site 3), and Callville Bay (site 6) are shown in figure 6. 11-KT is an important sex-steroid hormone responsible for spermatogenesis in male fish. Female carp from Callville Bay, the reference site, have median 11-KT concentrations that are about one-third of the concentrations in males; this is the normal ratio (Down and others, 1990). However, 11-KT concentrations in male carp from Las Vegas Bay are significantly lower (p less than 0.05) than those in male carp from Callville Bay. Low concentrations of sex steroids can cause premature sexual maturity, reduced gonad size, and reduced secondary sex characteristics (Munkittrick and others, 1992).

Blood-plasma samples from female carp collected from Las Vegas Wash had significantly higher 11-KT concentrations than female carp from Las Vegas and Callville Bays (p less than 0.05). Similarly high concentrations of 11-KT have been found in female flounder (*Platichthys fiesus*) exposed to PAH contaminated sediment, which caused premature gonadal development (Janssen and others, 1995).

Concentrations of an important female fish sex-steroid hormone, 17β-estradiol (E₂), in blood-plasma samples from
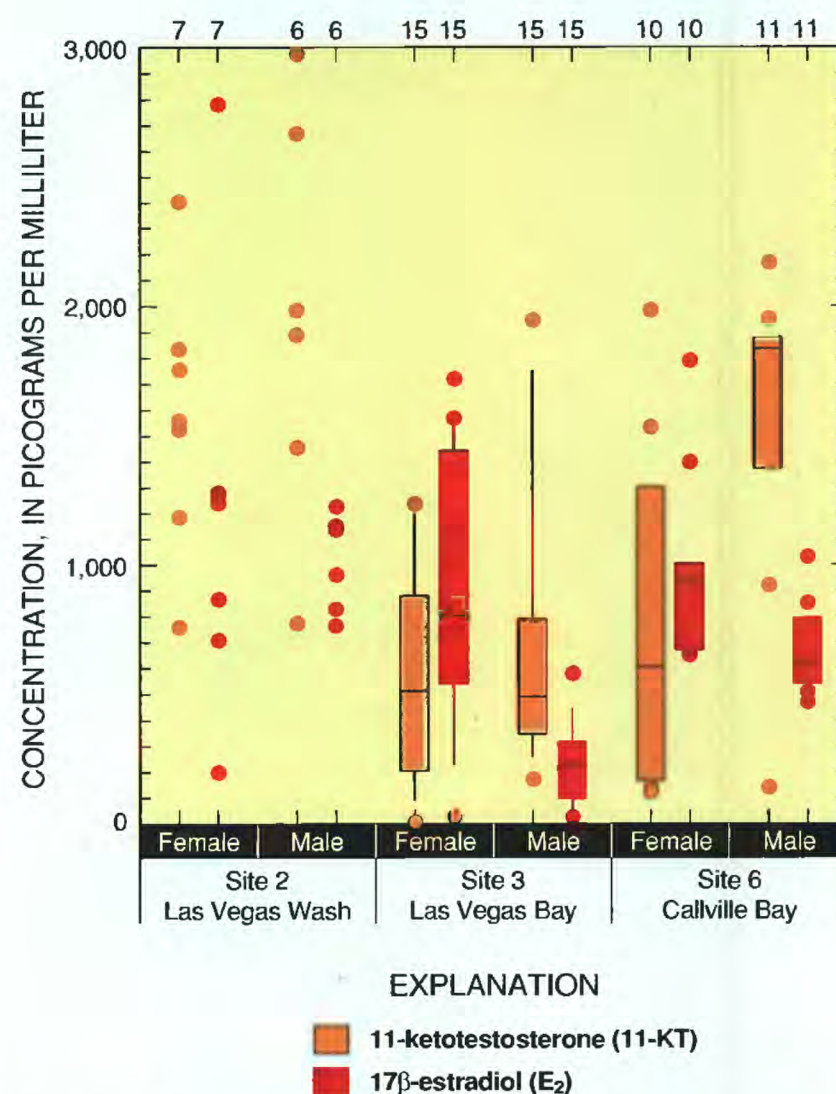

Figure 6. Concentrations of 11-ketotestosterone (11-KT) and 17β-estradiol (E₂) in blood-plasma samples from carp, Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, May 1995.

female and male carp collected from the Las Vegas Wash, Las Vegas Bay, and Callville Bay sites are shown in figure 6. Concentrations are significantly higher in females than in males for Las Vegas and Callville Bays (p less than 0.05), but male carp from Las Vegas Bay have significantly lower concentrations (p less than 0.05) than males from Callville Bay, the reference site. The low concentrations of 11-KT and E₂ measured in male carp from Las Vegas Bay indicate that their endocrine systems have been disrupted.

The ratio of E₂ to 11-KT concentrations (in picograms per milliliter) in blood plasma appears to be a sensitive marker of abnormal sex-steroid concentrations (Folmar and others, in press). During early development, specific E₂/11-KT ratios are necessary in males and females for proper sexual differentiation; altered ratios may result in incomplete gonad development (Hileman, 1994). Also, the balance between these two hormones in a fish determines its phenotype, which includes sexual characteristics, differentiation of the brain and behavior, and development of reproductive organs (Hunter and Donaldson, 1983).

E₂/11-KT ratios in blood-plasma samples from male carp are 1 or less for all but one sample (fig. 7), indicating a predominance of 11-KT compared to E₂. Female carp from Las Vegas and Callville Bays have ratios generally greater than 1, showing a predominance of E₂. The E₂/11-KT ratio of 1.0 appears to be an approximate division between females (E₂/11-KT greater than 1.0) and males (E₂/11-KT less than 1.0) on

the basis of measurements made for this study; however, female carp from Las Vegas Wash have ratios similar to those of males. These low ratios may indicate endocrine disruption and are caused by significantly higher 11-KT levels in female carp from Las Vegas Wash compared with females from Las Vegas and Callville Bays (p less than 0.05).

The most compelling evidence of endocrine disruption in this study is the presence of vitellogenin in blood-plasma samples from male carp from Las Vegas Wash and Bay, and significantly higher vitellogenin concentrations (p less than 0.05) in females from Las Vegas Bay compared with females from Callville Bay (fig. 8). Vitellogenin has no known function in male carp; its presence indicates that some type of estrogenic substance has bound to the estrogen receptor in the hepatopancreas of male carp from Las Vegas Wash and Bay.

Histologic analysis showed that all males had some spermatogenic activity and all females, except one, were in some vitellogenic stage. Gonad samples were evaluated for sexual-maturity level to ensure that observed differences in levels of sex hormones or vitellogenin were not caused by different levels of sexual maturity. The results indicate similar levels of sexual maturity among carp of the same gender collected at the three sampling sites. The normal appearance of all gonad samples histologically analyzed for this study indicates that endocrine disruption had not altered gonadal tissue structure. A life history study of carp in the area of study would be needed to determine if there are reproductive effects induced by the observed endocrine disruption.

Histological analysis revealed slight necrotic changes in hepatopancreas and kidney samples of all fish from Las Vegas Wash and Las Vegas and Callville Bays. These changes included multi-focal distributions of renal interstitial cells,
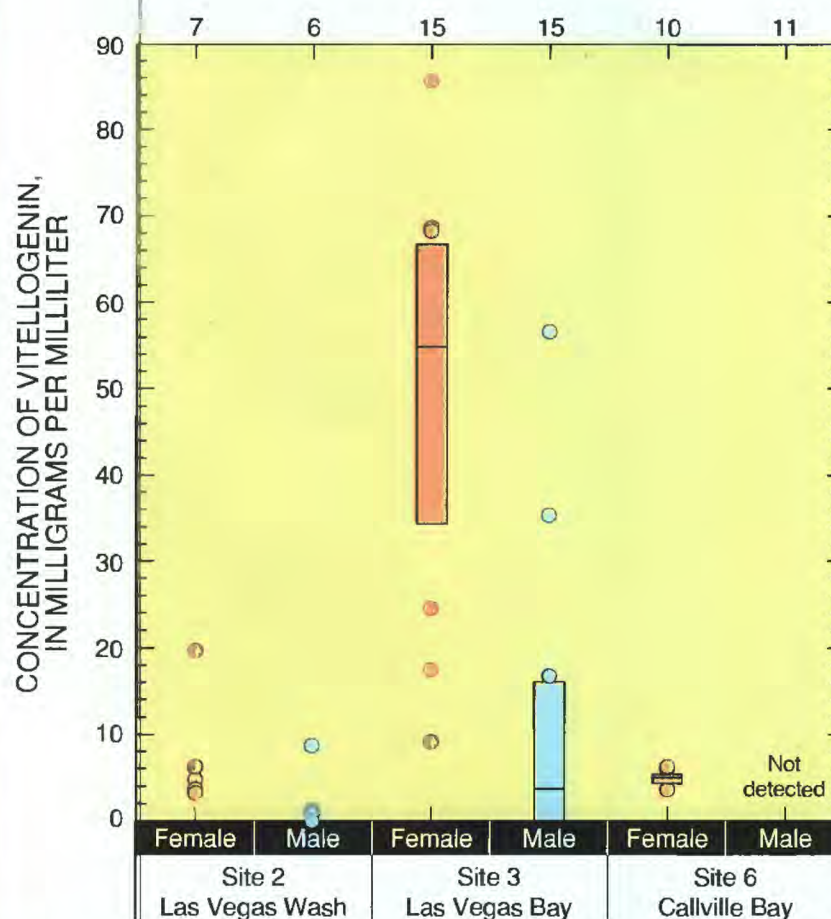
Figure 8. Concentrations of vitellogenin in blood-plasma samples from carp, Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, May 1995.

acinar cells, and hepatocytes filled with lipofuscin. Lipofuscin is an insoluble pigment associated with cells undergoing slow regressive changes. Also present were degenerative kidney tubules and glomeruli filled with hyaline deposits. The patterns and severity of necrosis, combined with the presence of apparently normal tissues in hepatopancreas and kidney samples, suggest long-term, subchronic exposures to a toxicant or combination of toxicants (J.S. Foott, U.S. Fish and Wildlife Service, written commun., 1995). One parasitic cyst was observed in the gill of a fish from Callville Bay. All other gill samples appeared normal. Abnormalities were not observed in lower intestinal tissues from any fish.

SUMMARY

The USGS, NPS, NBS, and USFWS investigated the occurrence of organochlorines and semivolatile industrial compounds in Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead. They also assessed endocrine disruption and the histologic effects to selected organs in carp (*Cyprinus carpio*) from those areas. Las Vegas Wash transports urban runoff and treated sewage effluent to Las Vegas Bay from the Las Vegas area. Callville Bay is upstream from Las Vegas Bay.

Numbers and combined concentrations of organochlorines were greatest in semipermeable-membrane-device (SPMD), bottom-sediment, and carp-tissue samples from Las Vegas Wash and Bay, and least in samples from Callville Bay. Concentrations of hexachlorobenzene, dacthal, trans-chlordane, cis-chlordane, trans-nonachlor, dieldrin, *p,p'*-DDD; *p,p'*-DDE, total PCB's, combined TCDD's, and combined TCDF's were greatest in the SPMD sample from Las Vegas Wash, less in Las Vegas Bay, and least in Callville Bay.
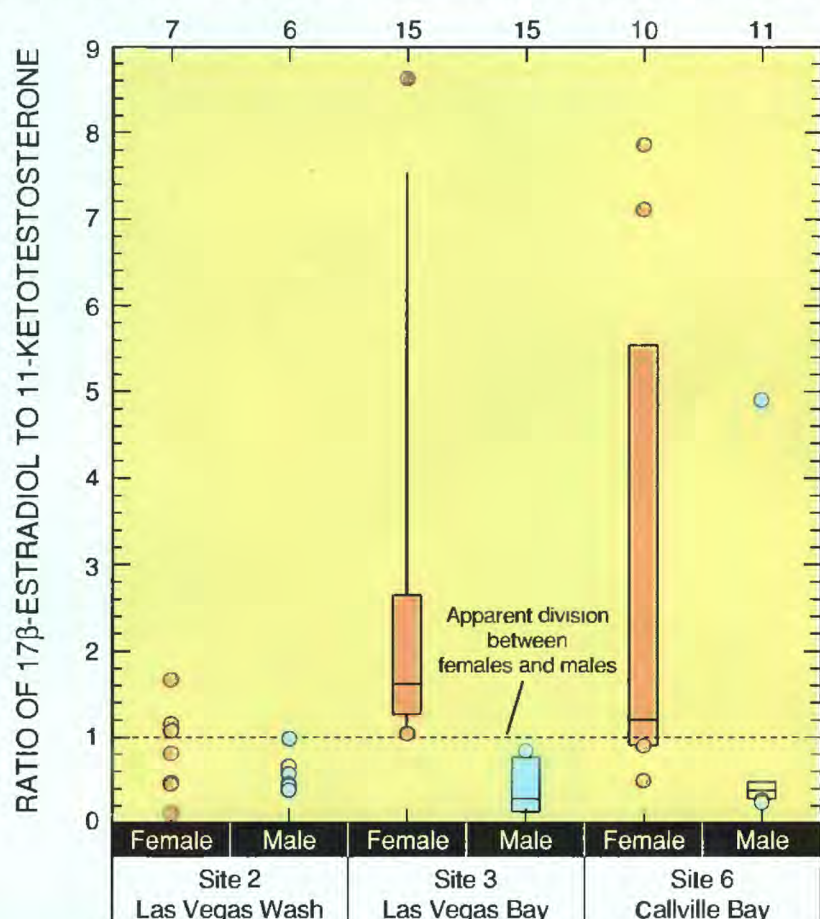
Figure 7. Ratio of 17β-estradiol to 11-ketotestosterone (E_2/11-KT) in blood-plasma samples from carp, Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, May 1995.

The DDT metabolites p,p'-DDD and p,p'-DDE were detected in bottom-sediment samples from all Las Vegas Bay sites, but were not detected in Callville Bay. Carp-tissue samples from Las Vegas Wash and Bay had significantly (p less than 0.05) higher combined concentrations of organochlorines, including DDT residues (sum of p,p'-DDT; p,p'-DDD; and p,p'-DDE), than samples from Callville Bay. Dioxins and furans were detected in bottom-sediment samples from Las Vegas Bay in combined concentrations that were an order of magnitude higher than those in samples from Callville Bay. OCDD's, OCDF's, and 2,3,7,8-TCDF were detected in bottom-sediment samples from the three Las Vegas Bay sites, but not in the sample from Callville Bay. These areal distributions indicate that Las Vegas Wash is a source of these organochlorines to Las Vegas Bay.

Results of a 2,3,7,8-TCDD toxic equivalent bioassay analysis of carp-tissue samples indicate the presence dioxins or furans with low toxic equivalents in Las Vegas Wash and Las Vegas and Callville Bays. This is consistent with the types of these compounds detected in SPMD and bottom-sediment samples.

The median concentration of DDT residues in cross-sectional carp-tissue samples from Las Vegas Wash (72 μg/kg, net weight) and Bay (120 μg/kg, wet weight) exceeded the risk-based human consumption limit of 60 μg/kg, wet weight of edible tissue, assuming consumption of more than 2 and 0 (zero) ounces per month, respectively, and a protection level against a 1×10^{-6} increase in cancer risk. Median concentrations of DDT residues and a mixture of compounds resembling Aroclor 1254 in carp-tissue samples from Las Vegas Wash and Bay exceeded selected risk-based consumption limits for chronic-systemic health effects. These results indicate that concentrations in edible portions of game fish may exceed some USEPA consumption limits.

Numbers and combined concentrations of PAH's in SPMD samples and concentrations of the individual compounds 2,3,6-trimethylnaphthalene; 4,5-methylenephenanthrene; phenanthrene; fluoranthene; and chrysene in outer reaches of Las Vegas and Callville Bays were similar to or higher than numbers and concentrations detected in Las Vegas Wash. Concentrations of PAH's in bottom-sediment samples from Las Vegas Wash and Bay were an order of magnitude higher than the sample from Callville Bay; 2,6-dimethylnaphthalene was detected at all Las Vegas Wash and Bay sites and pyrene was detected at all sites except site 5. These areal distributions indicate that Las Vegas Wash may be a source of these compounds for Las Vegas Bay, but also that there are perhaps more general sources of PAH's to Lake Mead.

Combined concentrations of phthalates were about an order of magnitude higher in bottom-sediment samples from Las Vegas Wash and Bay than in the sample from Callville Bay. The phthalates di-n-butylphthalate and diethylphthalate were detected in bottom-sediment samples from all Las Vegas Bay sites. The phenolic compound p-cresol was detected at high concentrations in bottom-sediment samples collected from Las Vegas Bay during 1992 (4,050 μg/kg, dry weight) and 1995 (930 μg/kg, dry weight), but was not detected in the 1995 sample from Callville Bay. Las Vegas Wash may be a source of these compounds.

Endocrine disruption in Las Vegas Wash and Bay, as compared to Callville Bay, is evidenced by lower 11-KT concentrations in blood-plasma samples of male carp from Las Vegas Bay (p less than 0.05) and higher concentrations in female carp from Las Vegas Wash (p less than 0.05), and lower concentrations of E_2 in male carp from Las Vegas Bay (p less than 0.05). Lower E_2/11-KT ratios in female carp from Las Vegas Wash (p less than 0.05) may indicate endocrine disruption. The most compelling evidence of endocrine disruption is the presence of vitellogenin (an estrogen-controlled egg protein normally found only in females) in blood-plasma samples from male carp from Las Vegas Wash and Bay, and elevated vitellogenin levels in female carp from Las Vegas Bay.

Results of histologic analyses of hepatopancreas and kidney samples from carp indicate slight necrotic changes in all carp from Las Vegas Wash and Las Vegas and Callville Bays. The patterns and severity of necrosis, combined with the presence of apparently normal tissues, are consistent with effects of long-term subchronic exposure to a toxicant or a combination of toxicants.

Many of the organochlorines and semivolatile industrial compounds detected in semipermeable membrane devices, bottom sediment, and carp tissue from Las Vegas Wash and Bay have been linked to endocrine disruption in fish by previous investigations of other areas. The observed endocrine disruption in carp from Las Vegas Wash and Bay could be due to the presence of these compounds.

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