APPENDIX I

FISH HEALTH ASSESSMENT

Appendix I

FISH HEALTH ASSESSMENT

Introduction

The CCH's Ocean Monitoring Program requires regular assessments of fish health to determine if there is an adverse effect from the Sand Island wastewater discharge on representative fish species. Assessments of physical abnormalities and diseases in rig-caught fish species is performed by CCH monitoring personnel and specialists under contract to address specific monitoring requirements and permit conditions. Visual examination of fish is specified because a number of physical abnormalities and diseases have gross external manifestations, such as epidermal papillomas and fin lesions in fish (Murchelano 1982).

Fish health studies usually target demersal species (such as flatfish) because they are particularly susceptible to physical abnormalities and diseases, which appear to be associated with contaminated sediments (Johnson et al. 1992, 1993; Moore et al. 1997; Myers et al. 1993, 1994; Stehr et al. 1997,1998). This is because they live in direct contact or in close association with sediments, many of them feeding either directly or indirectly on benthic infauna, which also may be contaminated with pollutants from wastewater particles and/or sediments. This has been particularly true to mainland estuarine and nearshore waters where conditions allow for accumulation of organics.

In Hawaii, particularly in the waters of Mamala Bay, sedimentation and accumulation of organic matter of a wastewater origin has not been observed to have any significant measurable impact on sediment quality. However, the CCH does perform histopathological examination of the livers of fish caught near the outfall (caught at depths of 160-270 feet (48.8 and 82.3 meters) and at control sites in Maunalua Bay (to the south of Diamond) (depths of 50 feet and 220 feet (15.2 and 36.6 meters) to determine if there are any signs of chronic effects on fish from wastewater disposal practices.

Purpose and Objectives

These fish health studies are conducted to answer the following questions:

1) Is the outfall an epicenter of disease (external and liver parasitism, epidermal tumors, fin and skin lesions, and liver lesions)?

2) Are chemical contaminants in sediments (or prey organisms) positively associated with the prevalence of certain liver lesions?

The CCH determines compliance with this monitoring requirement by assessing the external condition of each rig-caught fish and the internal health of selected fish taken live to the laboratory and necropsied. Assessments of external conditions include visual examinations to document abnormal growths, atypical color patterns, parasites, fin lesions, and other types of lesions.

In the laboratory, liver tissues from each specimen are evaluated for parasites and pathological conditions and a report prepared on an annual basis.

Pollution-Induced Fish Diseases

Fishes residing in proximity to sewage outfalls could be at an increased risk for pollution related diseases or stress induced influences from changes in water quality (ie low dissolved oxygen, toxic chemicals, etc.)(U.S. EPA, 1987).

The literature on the relationship of fish pathology to pollution in marine and estuarine environments has identified at least four grossly visible conditions as acceptable for immediate use in monitoring programs (Sindermann et al., 1980 and EPA, 1987) and which has been documented in associated with wastewater discharges or marine and estuarine pollution (EPA, 1987 and OCSD, 2002):

- Fin erosion
- Skin ulcers
- Skeletal anomalies
- Neoplasms (i.e., tumors).

Fin erosion is found in a variety of fishes from contaminated environments. It probably is the most frequently observed gross abnormality in polluted areas (Sindermann 1983). In demersal fishes, the dorsal and anal fins are the ones most frequently affected whereas in pelagic fishes, the caudal fin is the one primarily affected (U.S. EPA, 1987). The causes of fin erosion are unknown and likely complex. They may include chemical contaminants, low dissolved oxygen, and pathogens. Fin erosion has been induced in fishes after laboratory exposure to petroleum and PCBs (EPA, 1987).

Skin ulcers have been found in a variety of fishes from polluted habitat, and next to fin erosion, they are the most frequently reported gross abnormalities in polluted areas (Sindermann 1983). Prevalence of ulcers generally varies with season, and is often associated with organic enrichment. The primary cause of skin ulcers may be pathogenic organisms (e.g.*Vibrio* pp.) associated with pollution.

Skeletal anomalies frequently are more prevalent in fishes from polluted areas than in fishes from uncontaminated areas. Most observed skeletal anomalies involve the spinal column and include fusions, flexures, and vertebral compressions. Skeletal anomalies also include abnormalities of the head, fins, and gills. Skeletal anomalies have been induced in fishes after laboratory exposure to kepone and heavy metals (Sindermann et al. 1980).

Neoplasms or tumors have been found in elevated prevalence in a variety of polluted areas throughout the world (U.S. EPA, 1987). The most frequently reported grossly visible tumors include liver tumors, skin tumors (i.e., epidermal papillomas and/or carcinomas), and neurilemmomas. Liver tumors have been induced in fishes after laboratory exposure to a variety of chemicals. Two kinds of growths have been described as epidermal "papillomas" and pseudobranchial "tumors" in the literature (Sindermann et al. 1980). The predominant cell type in these growths is the presently unidentified X-cell which has been suggested is probably is a protozoan parasite, possibly an amoeba of the family Harmanellidae (Dawe 1981; Myers 1981). No relationship between the prevalence of these skin anomalies and pollution has been demonstrated conclusively.

Fish Histopathology Studies

Studying the diseases of fish around ocean outfalls utilizing histopathology was initiated in 1969 in studies of the Orange County Sanitation District outfall (old 20 meter outfall) in Southern California. The fish around the outfall were shown to have a high incidence of fin erosion and epidermal papillomas and other symptoms of severe stress. Other outfalls were also studied by the scientists of the Southern California Coastal Water Research Project (Mearns and Sherwood, 1974 and 1977). The National Oceanic and Atmospheric Administration had scientists who also initiated studies in Washington state which eventually were performed throughout the country incorporating fish histopathology into monitoring programs (NOAA Status and Trends Program).

Fish Diseases Associated with Contaminated Sites

Histopathological examination of fish, such as has been done by the CCH for fish caught near the Sand Island outfall is done to evaluate fish health and any signs of chronic disease. In national studies done by the National Oceanic and Atmospheric Administration as part of its Status and Trends program, liver neoplasms (cancerous tumors) were found in 10 fish species collected
from 1984 to 1988 from sites near urban centers along the west and northeast coasts where sediments were highly contaminated with pesticides or hydrocarbons (Turgeon et al. 1992). Scientists concluded that the contaminants most likely to be factors in the development of these tumors were the PAHs, PCBs, and DDTs (Myers et al. 1993).

Tumors In Fish

To date, incidences of cancerous tumors are generally low in fish from U.S. coastal waters, other liver disease conditions, some of which may progress to neoplasms, occur more frequently in areas where contaminants are high. Neoplasms and pre-neoplasms (pre-cancerous tumors) were found in up to 15% of the winter flounder from sites in Boston Harbor (Murchelano and Wolke 1991). Along the west coast, neoplasm incidences are well below 10% in most fish species (Myers et al. 1993). Relatively high incidences of nontumorous disease conditions occur in fish from contaminated sites. For example, in English sole (*Parophrys vetulus*) from Elliott Bay, Washington, incidences of 42% for specific degeneration and necrosis (SDN) of liver

cells and 13% proliferative disorders (cells duplicating out of control) have been recorded; and in white croaker from San Pedro Outer Harbor, California, 22% SDN and 7% for proliferative disorders have been found (Varanasi et al. 1989; Myers et al. 1993). At Morris Cove, a highly contaminated site in New Haven, Connecticut, up to 90% of the cells in winter flounder livers have been found to be vacuolated cells (large areas of apparently empty, nonfunctioning cells; Gronlund et al. 1991).

Fin Erosion

Although fin erosion (fish with reduced fins or in extreme stages of disease with no fins) has been found in all species at all sites, this condition is still unusual, except in a few highly contaminated areas. Eroded fins occurred in 27% of the black croaker (*Cheilotrema saturnum*) and 22% of barred sand bass (*Paralabrax nebulifer*) from the West Harbor site in San Diego Bay, California (McCain et al. 1989). Up to 90% of Atlantic croaker, 100% of sand seatrout (*Cynoscion arenarius*), and 17% of spot sampled from the Houston Ship Channel at Green Bayou, Texas, experienced fin loss due to disease

Reproductive impairment occurred in fish from Eagle Harbor and Duwamish Waterway in Puget Sound, San Francisco and San Pedro bays, and in Morris Cove. Significantly lower levels of estradiol (a reproductive hormone) and vitellogenin (yolk protein critical to the development of fertile eggs for reproduction) have been found in English sole from contaminated sites in Puget Sound than those at relatively clean sites (Johnson et al. 1989). Also, a significant proportion of fish from contaminated sites failed to produce yolked eggs and undergo normal ovarian development. Moreover, fewer English sole spawned from the Duwamish Waterway (54%) in comparison with those from Port Susan during the 1987 and 1988 reproductive seasons (Casillas et al. 1991).

White croaker from a site near Los Angeles and kelp bass (*Paralabrax clathratus*) from San Pedro Bay had lower reproductive success than those from less contaminated sites at Dana Point and Santa Catalina Island (Cross and Hose 1989). In this study, the percentage of spawning fish was 24%-68% lower, batch fecundity (number of eggs produced) was 36%-44% lower, and the proportion of eggs fertilized was 14%-45% lower at the contaminated site. Gonadally mature female starry flounders from an urbanized central San Francisco Bay site off Berkeley had a reduced proportion of floating eggs and poorer fertilization success than those captured at a site in northern San Pablo Bay (Spies and Rice 1988). In Long Island Sound, embryo abnormalities were most frequent and hatching success was lowest in female winter flounders from more contaminated sites near Milford and New Haven; larvae were smallest off Deer Island, a highly contaminated site in Boston Harbor (Nelson et al. 1991).

Thus, there is evidence from collections of benthic fish at estuarine sites with contaminated sediments that lower reproductive success may occur and this could have long-term effects on spawning populations of some sensitive species, particularly flatfish species.

Chemically Induced Liver Lesions in Fish

As discussed above, organic chemicals, particularly hydrocarbons and persistent fat-soluble organics like organochlorine pesticides, PCBs, and (polyaromatic hydrocarbons or PAH's) have been found to be associated with diseases in fish. Most often these situations have occurred in estuarine areas or enclosed water bodies with highly contaminated sediments. However, the potential for detecting the effects of chemicals on fish can be determined using histopathology to examine liver (hepatic) tissues from fish. This monitoring tool has been applied in some instances to the 301(h) permittees. The CCH initiated the use of this monitoring tool and used the EPA protocols (U.S. EPA, 1987) to undertake studies to determine fish health.

The list of potential chemicals that have been found in laboratory studies to induce liver lesions is quite large. Most often however, these studies employ high doses and the levels to which the fish are exposed are many-fold greater than might be measured in the environment, particularly in well-mixed open ocean waters. However, the list does include some of the chemicals which have been found in fish muscle tissue in the fish in Mamala Bay (See Appendix H). This includes DDT and PCBs (although the particular PCB's found in the fish in Mamala Bay are not the 1260 and 1254 fractions most often found to be responsible for inducing lesions).

TABLE I -1
CHEMICALS THAT HAVE INDUCED HEPATIC LESIONS
IN FISHES FOLLOWING LABORATORY EXPOSURE

Organochlorine insecticides
Chlordane
DDT
Dieldrin
Endosulfan
Endrin
Heptachlor
Hexachlorocyclohexane (beta isomer, lindane byproduct)
Kepone
Lindane
Methoxychlor
Toxaphene

Organochlorine herbicides
Dichlobenil
Dowicide G
2,4-D
Kuron (silvex)
Tordon 101 (picloram and 2,4-D as amine salts)
Tordon 22K (picloram, potassium salt)

Industrial organochlorine compounds
PCB-Aroclor 1248
PCB-Aroclor 1254
PCB-Miscellaneous
Carbon tetrachloride
Monochlorobenzene

Organophosphate insecticides
Abate (temphos)
Diazinon (Spectracide)
Dimethoate (Cygon)
Dursban (chlorpyrifos)
Dylox (trichlorfon)
Malathion
Methyl parathion

Carbamate insecticides
Aldicarb (Temik)
Carbaryl (Sevin)
Propoxur (Baygon)

Miscellaneous herbicides
Acrolein
Amitrole-T
Dinoseb
Diquat
Hydrothol 191
Paraquat-CL

Fossil-fuel related compounds
Benzo(a)pyrene (BaP)
Crude oil-whole
Crude oil-water soluble fraction
7-12 Dimethylbenz(a)anthracene (DMBA)
Oiled sediments

Chemotherapeutic agents
Copper sulfate
Diethylstilbestrol (DES)
Sulfamethazine
Thiabendazole

Mycotoxins
Aliatoxin B_1 (AFB)
Aflatoxin G_1 (AFG)
Aflatoxin M_1 (AFM)
Aflatoxin Q1 (AFQ)
Afl atoxicol (AFL)
Ochratoxin A + B
Sterigmatocystine
Versicolorin A

Plant derivatives
Cycad nut meal
Cycasin
Cyclopropenoid fatty acids (CPFA)
Gosypol
Methylazoxymethanol acetate (MAMA)
Pyrrolizidine alkaloids
Tannic acid

Nitroso- compounds
N,N'-dinitrosopiperazine (DNP)
N-nitrosodiethylamine (DEN)
N-nitrosodimethylamine (DMN
N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)
N-nitrosomorpholine (NM)

Miscellaneous nitrogenous compounds
2-Acetylaminofluorene (2-AAF)
o-Aminoazoto]uene (o-AAT)
Ammonia
Benzidine
Carbazone
p-Dimethylaminoazobenzene (DAAB
Thiourea
Urethane

Miscellaneous organic and organometallic compounds
Bis(tri-n-butyltin) oxide
Dimethylsulfoxide (DMSO)
Methylmercuric chloride
Nitro-3-(trifluoromethyl)phenol
Phenol

Inorganic compounds
Arsentates(sodium and disodium)
Cadmium chloride
Cupric sulfate
Lead nitrate
Mercuric chloride

Source: U.S. EPA, 1987.

Histopathological Evaluation of Fish

Histopathological analyses of fish livers are performed because:

> Pollutants tend to be most highly concentrated in livers;
> Livers are sites of metabolism for certain pollutants [i.e., chlorinated pesticides such as DDT and chlordane and polychlorinated biphenols (PCBs)]and the metabolic products of pollutants are known to have mutagenic and carcinogenic properties (Gmur and Varanasi 1982);
> Certain liver lesions are thought to be induced by contaminants and thus are useful indicators of contaminant exposure (as explained below); and
> Liver disease may occur in fish that have no external manifestations.

Some liver pathologies have been shown to be positively correlated with exposure to chemical contaminants (typically, related to fish age and/or size) and, therefore, are useful indicators of contaminant exposure effects (Bodammer and Murchelano 1990; Hinton and Lauren 1990; Hinton et al. 1992; Johnson et al. 1992; Moore et al. 1997: Myers et al. 1993, 1994; Stehr et al.1997,1998). Pathologies of particular concern are neoplasms (tumors) and cholangioma along with preneoplastic foci of cellular alteration (FCA), and severe hydropic vacuolation (SHV) of hepatopancreatic parenchyma. These changes are observed microscopically by trained specialists (fish pathologists) after the tissues are preserved, stained, imbedded in paraffin blocks, thinly cut and mounted on glass slides for microscopic examination.

The study of the livers of fish was started in the late 1960's when it was observed that English sole in San Francisco Bay had abnormal livers and tumors (cancer) (Cooper, 1969). Since that time a lot of study has gone into studying various flatfish and looking at the pathogenesis of the liver diseases experiences in the English sole. The following major hepatic lesions that are thought to be related to or associated with the histogenesis of liver neoplasms in English sole, which has among the most complete studies on flatfish histogenesis done (Meyers, et al. 1987):

- Nonspecific necrotic lesions
  - Hepatocellular coagulation necrosis
  - Liquefactive necrosis
  - Hydropic degeneration
  - Pyknosis
  - Hyalinization
  - Cystic parenchymal degeneration

- Specific degenerative conditions
  - Nuclear pleomorphism
  - Megalocytic hepatosis

- Nonneoplastic proliferative conditions
  - Nonhyperplastic hepatocellular regeneration

- Foci of cellular alteration
  - Eosinophilic foci
  - Basophilic foci
  - Clear cell or vacuolated cell foci
  - Hyperplastic regenerative foci

- Neoplasms
  - Liver cell adenomas
  - Hepatocellular carcinomas
  - Cholangiomas
  - Cholangiocellular carcinomas
  - Mixed carcinomas.

This overview of fish diseases is provided for background and understanding of some of the terms and conditions used in evaluating fish health and what results have been found in other areas where such studies have been performed.

Results and Discussion of Other Pacific Coast Fish Health Assessments

External Parasites in Fish Near Outfalls

Changes in parasite prevalence are a species-specific population phenomenon and changes in the incidence may reveal altered levels of environmental stress and/or changes in resistance to these stresses (OCSD, 2001). However, as evidenced in extensive studies in southern California near a large 301(h) permitted wastewater discharge, the overall prevalence of external parasitism [(for fish collected off Orange County, California near the Orange County Sanitation District's outfall which is the largest 301(h)-permittted discharge (240 mgd) during July 2000 and January 2001] was 0.65% (161 out of 24,869 individuals), up from 0.11% from the previous year (OCSD 2001). Eye copepod parasites (*P. cincinnatus*) were the most prevalent external parasite accounting for most of the observed incidences (0.57%) and were only found on Pacific sanddabs (140). The prevalence of this eye parasite for Pacific sanddabs is well below the average of 1.4% reported for the Southern California Bight (SCB)(Perkins and Gartman 1997). Similarly, the 1994 Southern California Bight Pilot Project (SCBPP) found eye parasites only on Pacific sanddabs with a prevalence of 1.1% (47 out of 4123) (Allen et al. 1998).

Leeches and other types of external body parasites included copepods and other small unidentifiable parasites are also observed on occasion.There was no spatial pattern relative to the Orange County outfall for the prevalence of parasites. Over a 16 years period, external parasites were observed in 0.11 to 1.0% of the fish specimens (OCSD 1996, 1997 and 2001). Compared with other areas of the SCB (Mearns and Sherwood 1977; Robinson 1982;), the incidence of parasitism in the OCSD study area is low. Studies have also shown little evidence of a chemical contaminant relationship for parasitism (Johnson et al. 1993). Fin lesion is rare, and was found on a single California scorpionfish at an outfall monitoring station in 2001.

Fin Lesions in Fish Near Outfalls

Over the past 16 years, studies of fish have measured frequencies of fin lesions ranging from zero to 0.11% (OCSD 1997, 1998, 1996a, 2000). These indices are very low compared with the 9% incidence of fin lesions among more than 170,000 individual fish collected by otter trawl on the Palos Verdes shelf from 1971–1981 (Cross 1985), and results from other studies in the Souther California Bight (SCB) (OCSD 1996, Mearns and Sherwood 1974, 1977; McDermott-Ehrlich et al. 1977; Sherwood and Mearns 1977; Cross 1982; Allen et al. 1998). More recently, the 1994 SCB Pilot Project (similar in intent to the regional monitoring effort now being done in Mamala Bay) collected 18,912 fish and found one fin lesion on a spotted turbot and 7 body lesions on 5 different species giving an overall prevalence of 0.04%. Thus, there has been a dramatic reduction over the past three decades in these types of lesions throughout the SCB, including the OCSD study area. No such fin lesions have been found on fish caught near the Sand Island outfall.

Tumors in Flatfish Near Outfalls

Epidermal tumors in Dover sole which were once prevalent have also declined dramatically and while the SCBPP project found only 1% prevalence throughout the SCB, this condition has not been observed in Dover sole from the Orange County study area in over three years. The present, low levels of epidermal tumors and fin lesions are consistent with the relatively low contaminant concentrations in bottom sediments and fish tissues . The low tumor incidences are also expected based on present low mass emissions from major outfalls, and improved source control and treatment practices that have occurred over the past two decades in southern California (OCSD 1997, 1998, 1996a, 2000). No fish with tumors have been caught to date near the Sand Island outfall or in reference areas.

Liver Parasitism

A variety of hepatic (liver) parasites, including biliary myxidial spores, coccidia, unidentified helminthic parasites, nematodes, trematodes, and larval canthocephalans, otherwise known as "mesenteric acanthocysts", can typically be found in fish.

Prevalence of liver parasites appears to be species-specific and has ranged from a few percent in some species to 100% in chub mackerel (*Scomber japonicus*) (OCSD 1996, 1997, 1998a). The interest in liver parasites stems from the possibility that stressful environmental conditions might make individual fish more susceptible to parasitism. Thus, the spatial pattern of parasite prevalence can be evaluated to determine whether the wastewater discharge may be contributing to parasite prevalence.

Coccidia protozoans are intracellular parasites of epithelial cells of annelids, mollusks, arthropods, and vertebrates. In fishes, they are often found in the bile ducts, liver, kidneys, and sometimes in the testes.

Coccidia typically have most heavily parasitized white croaker, an important target

species, having an average prevalence between 1998-2000 of 31.9%. Myxosporida represent another type of parasitic protozoan, which almost exclusively are found in fishes. Myxidial and their spores inhabit the hollow organs or live in the connective tissues of liver and kidneys and are mostly harmless to the host, but can cause damaging tumor-like masses. This type of parasite was most prevalent in English sole, parasitizing 15.6% of the specimens examined.

Trematodes are parasitic flatworms that attach to the host by means of suckers. The digenetic trematodes are responsible for many types of detrimental conditions in their hosts, including liver, lung, and blood flukes and tapeworms. Trematodes tend to be rare

Liver Pathology in Fish Near Outfalls

The prevalence of certain types of liver lesions have been shown to be positively correlated with exposure to chemical contaminants and, therefore, are useful bioindicators of exposure and environmental stress (Bodammer and Murchelano 1990; Hinton and Lauren 1990; Hinton et al. 1992; Johnson et al. 1992; Moore et al. 1997: Myers et al. 1993, 1994; Steer et al. 1997, 1998). Of the 392 fish livers evaluated from July 2000, 12 specimens (3.1%) had severe liver pathologies. Severe liver pathology is herein defined as those lesions related to disease and/or contaminant exposure, including neoplasms (cholangiocellular carcinoma, cholangioma, and liver cell adenoma), foci of cellular alteration (basophilic foci, clear cell foci, and esonophilic foci), and hydropic vacuolation, which pertains only to white croaker.

Other types of liver lesions are common, but these lesions may have multiple causes, including environmental contaminants, normal aging, infections, and parasite invasion. Classes of these type of lesions includes non-neoplastic proliferative lesions (biliary hyperplasia, cholangiofibrosis, hepatocellular regeneration, and parenchymal fibrosis), unique or specific degenerative/necrotic condition [hepatocelluar nuclear pleomorphism, and megalocytic hepatosis], and nonspecific necrotic lesions unassociated with visible infectious agents (hepatocellular coagulative necrosis, hepatocellular hyalization, hepatocellular hydropic degeneration, hepatocellular liquefactive necrosis, and non specific vacuolation). All liver lesions are considered detrimental to the health of the individual, but not all lesions are environmentally induced.

Of the 430 white croaker collected for the SPS in August 1999 and March 2000, 11 (2.6%) specimens were found with cholangiocellular carcinoma or cholangioma. None of the 26 specimens collected in July 2000 showed these lesions. This is in contrast to 1995 and 1996 when 5.3% and 4.3%, of the white croaker were found with these types of lesions, respectively.

Subsequent years have been very low with 0% in 1997, 0.7% (1 out of 143), and only one specimen caught in 1999 and this was without tumors. The overall prevalence of significant lesions has been decreasing since 1993 (12.6%) and suggests an improving trend. However, this apparent change in lesion prevalence can vary based on differences in the size/age of the individual collected. Annual changes in prevalence of

SHV are influenced by several factors, such as the species sampled, specimen size, sex, and collection location, in addition to possible contaminant exposure. SHV in white croaker decreased from 32% in 1994, to 8.7% in 1997 and to 2.6% in 1998; but increased in the August 1999 and March 2000 samples to 9.3%. This condition was not observed in any white croaker collected in July 2000.

These recent values are comparable to the approximately 8% prevalence for this lesion reported from Dana Point, a NOAA Status and Trends reference area (Stehr et al. 1998).

An analysis of histopathology data from 1986–1999 near the OCSD outfall revealed that the three target species collected near the outfall (within the zone of initial dilution by rig fishing and the nearest otter trawl station [T1]) had the highest prevalence of severe liver pathology within the study area. These findings suggested that a spatial pattern may exist for fish pathology relative to the outfall area. While prevalence and type of liver lesions tended to be species specific, in all cases larger and presumably older fish had a greater prevalence of severe lesion types (e.g., OCSD 1996, 1999; Myers et al. 1993,1994; Stehr et al. 1998). Furthermore, since larger fish tend to be found at the outfall, it would be expected that the outfall area would have a higher prevalence of liver pathology. However, because growth rates are highly variable, age is a better indicator of potential exposure than fish length. Consequently, age/lesion prevalence regressions were established for white croaker and tested to determine whether there were differences in the age of lesion onset (i.e., the regression intercept) or the rate of lesion acquisition (i.e., the slope of the regression) at the outfall compared to the farfield area. The results of this analysis found that lesion prevalence increases significantly with age and there were no sex or location differences for SHV and neoplasms. Thus, the outfall has the same prevalence for these types of lesions as the farfield area indicating that the outfall area is not a disease epicenter for these lesion types. Significant sex/location effects were found for FCA lesion types and all Severe Lesions combined, but these results indicate that older females in the farfield have a greater prevalence for FCA than males or females at the outfall and farfield males. Additionally, middle-aged males at the outfall develop Severe Lesions at a later age than elsewhere and there were no sexual differences for Severe Lesion types. Thus, the outfall is not a disease epicenter for liver pathology in white croakers. A similar type of analysis was conducted for bigmouth sole and hornyhead turbot based on size-pathology relationships and a similar conclusion was reached that the outfall was not a disease epicenter..

The specific studies conducted to examine the fish health of fish caught near the Sand Island outfall diffuser and at control sites removed from any potential sources of contaminants is discussed below.

Sand Island Outfall Fish Liver Histopathology Studies

To address this potential concern, histopathological studies of fish were initiated in 1992 to accompany visual observations being made for gross abnormalities of rig-caught fish collected for bioaccumulation studies. In 1992, thirty specimens of three

species of fishes were collected from the area around the Sand Island deep water diffuser. These fishes were examined for gross necropsy and liver histopathology by J. Brock (1993). The three species of fishes were the humuhumu mimi or bridled triggerfish (*Sufflamen fraenatus*), the blue-lined snapper or taape (*Lutjanus kasmira*) and the big-eyed scad or akule (*Selar crumenophthalmus*). Unlike other areas where these studies are done, Hawaii does not have a large number or abundance of flatfish species, and thus the target species for the studies are those species in most abundance near the outfall and are of commercial and recreational importance to the local fishery.

The food habits of *Sufflamen fraenatus* are unknown but other related balistids feed on small, heavy-bodied invertebrates such as crabs, mollusks and echinoids by use of their powerful jaws and heavy dentition (Hobson 1974).

Since the first study was completed in 1992 (Brock, J.A., 1993), several others have been completed to evaluate fish health (Brock, J. A., 1999 and 2000 and Work T. M., 2001 and 2003). The results are submitted to the EPA and State DOH and reported in the Sand Island WWTP Annual Assessment Reports. Since the new permit was issued in late 1998, the fish health assessment and bioaccumulation studies of fish have focused on two species the blue-lined snapper or tape (*Lutjanus kasmira*) and the big-eyed scad or akule (*Selar crumenophthalmus*)

Results of Year 2001 Fish Histopathology Study

The abstract from the 2001 Fish Histopathology Report is presented below:

"Fish liver histopathology is an important biological tool used to assess fish for exposure to pollution because fish collected from polluted environments may have neoplasms in the liver. In 2001, gross necropsy and fish liver histopathology were conducted on 30 specimens each of *Lutjanus kasmira* and *Selar crumenophthalmus.* The fish were collected live near the terminus of the Sand Island Ocean Outfall (20 fish) and near reference stations FR 1 (20 fish) and FR2 (20 fish) in Maunalua Bay. Two *L. kasmira* from the outfall station had either bile duct hyperplasia or periportal fibrosis, whereas the remaining fish had combinations of acute inflammation, suspect myxosporidia in the bile duct epithelium, and atrophy of liver cells. Putative myxosporidia, acute inflammation, encysted metazoans, and liver atrophy were major lesions seen in *L. kasmira* from reference station FR1. Putative myxosporidia, acute necrosis and hemorrhage, emphysema, and acute and chronic inflammation were major lesions seen in *L. kasmira* from reference station FR2. Hemorrhage and acute necrosis were commonly seen in *S. crumenophthalmus* from all sites; emphysema and focal chronic inflammation were less commonly seen. Neoplastic changes were not seen. Wild fish have many parasites (protozoa, nematodes, cestodes), some of which migrate through the liver and most of which are species-specific in their life cycles. Based on widespread presence of such parasites in *L. kasmira,* it is likely that they were responsible for the bile duct hyperplasia and fibrosis seen in the specimens from the outfall station. The emphysema, hemorrhage, and acute necrosis were rapid changes most likely due to the decompression trauma the fish received during capture.

Granulocytic infiltrates were mild and considered nonspecific. Gross or microscopic evidence of neoplasia was not found in the 60 fish evaluated."(Work, 2002)

Results of Year 2002 Histopathology Study

The abstract of the 2002 fish histopathology report is presented below:

"Fish liver histopathology is an important biological tool used to assess fish for exposure to pollution because fish collected from polluted environments may have neoplasms in the liver. In 2002, gross necropsy and fish liver histopathology were conducted on 90 specimens. Twenty specimens each of *Lutjanus kasmira* and *Selar crumenophthalmus* were collected live near the terminus of the Sand Island Ocean Outfall and at reference stations FR1 and FR2 in Maunalua Bay in September. An additional cohort of 10 specimens each of *Lutjanus kasmira, Selar crumenophthalmus,* and *Myripristis berndti* were collected live at reference station FR2 in October. At all stations, melanized macrophages centers were the most common change seen in the liver of both *S. crumenophthalmus* and *L. kasmira* individuals. Hemorrhage, acute necrosis, and lymphoid infiltrates were other changes seen in individuals of all three species of fish. Emphysema was noted only in *L. kasmira* specimens. An unidentified metazoan was noted in one *L. kasmira* specimen and one *M. berndti* specimen from the October sampling at reference station RF2 Coccidial infections were noted in small numbers of *S. crumenophthalmus* from the outfall and reference stations sampled in September but not at the reference station sampled in October. Neoplastic changes were not seen. Wild fish have many parasites (protozoa, nematodes, cestodes)? some of which migrate through the liver and most of which are species-specific in their life cycles. The emphysema and hemorrhage and acute necrosis were rapid changes most likely due to decompression trauma the fish received during capture. Lymphoid infiltrates were mild and considered nonspecific. Gross or microscopic evidence of neoplasia was not found in the 90 fish evaluated." (Work, 2003).

Discussion of Sand Island Fish Histopathology Results

The fish caught in the deep water near the outfall are subjected to acute decompression trauma during capture. Despite this, City and County of Honolulu personnel are able to keep the specimens alive for delivery to the Halawa necropsy facility several hours later making for ideal conditions for euthanization and subsequent necropsy.

In the examination of the fish, neoplastic changes (tumors) were not seen. When present, however, neoplastic changes are characterized by uncontrolled cell growth, aberrant cytoplasmic and nuclear morphology, rapid cell division (mitotic figures), displacement of adjacent normal tissue, presence or absence of encapsulation, hemorrhage, necrosis, and inflammation.

The hemorrhage, acute hepatocellular necrosis (liver damage), and emphysema were acute changes and most likely reflected the decompression trauma the fish received during capture and during the holding period prior to necropsy. Given the location of

capture of the fish in deep water, which necessitates their rapid decompression during retrieval from the bottom, these tissue changes are an expected finding.

Other lesions were considered incidental. Inflammatory changes (both acute ones manifested by granulocytes and chronic ones manifested by mononuclears) were mild and nonspecific and could have been caused by a variety of etiologies, including infectious, toxic, or other type of agents that can injure the tissue. Hepatocellular vacuolation could be an indication of physiologic storage of lipid or glycogen, or, in extreme cases, metabolic or toxic anomalies that can lead to damage to cytoplasmic organelles.

Myxosporidians and metazoans were rarely seen. Myxosporidians have complex life cycles that involve specific fish and invertebrate hosts. Subclinical infection by one or more myxosporidians is very common in wild fish. Additionally, wild fish harbor many other parasites (trematodes, nematodes, cestodes, various protozoa), some of which migrate through the liver and most of which are species-specific in their life cycles and thus are unlikely related to the effluent.

Conclusions

All the histopathological studies done on fish to date have shown that the two local species of commercial and recreational importance to local fishermen are healthy. There has been no evidence of acute or chronic disease symptoms which would indicate that the Sand Island outfall is a disease epicenter. There is no evidence of fin erosion, tumors, increased parasitism, ambicoloration, vertebral abnormalities, or other signs of disease. All fish appear to be healthy.

References

Note that all references cited are listed in Section IV of the application.

Method 614

The Determination of Organophosphorus Pesticides in Municipal and Industrial Wastewater

Method 614

The Determination of Organophosphorus Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

1.1 This method covers the determination of certain organophosphorus pesticides. The following parameters can be determined by this method:

Parameter	*STORET No.*	*CAS No.*
Azinphos methyl	39580	86-50-0
Demeton	39560	8065-48-3
Diazinon	39570	333-41-5
Disulfoton	39010	298-04-4
Ethion	–	563-12-2
Malathion	39530	121-75-5
Parathion ethyl	39540	56-38-2
Parathion methyl	39600	298-00-0

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.

1.3 The method detection limit (MDL, defined in Section 15) for several parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in Method 617. Thus, a single sample may be extracted to measure the parameters included in the scope of both of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under gas chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the

Table 1. Chromatographic Conditions and Method Detection Limits

Parameter	*Retention Time (min)*		*Method Detection Limit (µg/L)*
	Column 1	*Column 2*	
Diazinon	1.8	1.8	0.012
Disulfoton	1.9	2.1	ND
Demeton	2.3	2.1	ND
Parathion-methyl	2.5	3.7	0.012
Malathion	2.9	3.9	ND
Parathion-ethyl	3.1	4.5	0.012
Ethion	6.8	9.1	ND
Azinphos-methyl	14.5	29.9	ND

ND = Not determined

Column 1 conditions: Gas-Chrom Q (100/120 mesh) coated with 3% OV-1 packed in a glass column 1.8 m long by 4 mm ID with nitrogen carrier gas at a flow rate of 60 mL/min. Column temperature, isothermal at 200°C. A flame photometric detector was used with this column to determine the MDL.

Column 2 conditions: Gas Chrom Q (100/120 mesh) coated with 1.5% OV-17/1.95% QF-1 packed in a glass column 1.8 m long by 4 mm ID with nitrogen carrier gas at a flow rate of 70 mL/min. Column temperature, isothermal at 212°C.

METHOD 200.8

DETERMINATION OF TRACE ELEMENTS IN WATERS AND WASTES BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

Revision 5.4
EMMC Version

S.E. Long (Technology Applications Inc.), T.D. Martin, and E.R. Martin - Method 200.8, Revisions 4.2 and 4.3 (1990)

S.E. Long (Technology Applications Inc.) and T.D. Martin - Method 200.8, Revision 4.4 (1991)

J.T. Creed, C.A. Brockhoff, and T.D. Martin - Method 200.8, Revision 5.4 (1994)

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268
METHOD 200.8

DETERMINATION OF TRACE ELEMENTS IN WATERS AND WASTES BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

1.0 SCOPE AND APPLICATION

1.1 This method provides procedures for determination of dissolved elements in ground waters, surface waters and drinking water. It may also be used for determination of total recoverable element concentrations in these waters as well as wastewaters, sludges and soils samples. This method is applicable to the following elements:

Analyte		Chemical Abstract Services Registry Number (CASRN)
Aluminum	(Al)	7429-90-5
Antimony	(Sb)	7440-36-0
Arsenic	(As)	7440-38-2
Barium	(Ba)	7440-39-3
Beryllium	(Be)	7440-41-7
Cadmium	(Cd)	7440-43-9
Chromium	(Cr)	7440-47-3
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Lead	(Pb)	7439-92-1
Manganese	(Mn)	7439-96-5
Mercury	(Hg)	7439-97-6
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4
Thallium	(Tl)	7440-28-0
Thorium	(Th)	7440-29-1
Uranium	(U)	7440-61-1
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

Estimated instrument detection limits (IDLs) for these elements are listed in Table 1. These are intended as a guide to instrumental limits typical of a system optimized for multielement determinations and employing commercial instrumentation and pneumatic nebulization sample introduction. However, actual method detection limits (MDLs) and linear working ranges will be dependent on the sample matrix, instrumentation and selected operating conditions. Given in Table 7 are typical MDLs for both total recoverable determinations by "direct analysis" and where sample digestion is employed.

TABLE 7: METHOD DETECTION LIMITS

AMU Element	Scanning Mode[1] Total Recoverable Aqueous µg/L	Scanning Mode[1] Total Recoverable Solids mg/kg	Selection Ion Monitoring Mode[2] Total Recoverable Aqueous µg/L	Selection Ion Monitoring Mode[2] Direct Analysis[3] Aqueous µg/L
27 Al	1.0	0.4	1.7	0.04
123 Sb	0.4	0.2	0.04	0.02
75 As	1.4	0.6	0.4	0.1
137 Ba	0.8	0.4	0.04	0.04
9 Be	0.3	0.1	0.02	0.03
111 Cd	0.5	0.2	0.03	0.03
52 Cr	0.9	0.4	0.08	0.08
59 Co	0.09	0.04	0.004	0.003
63 Cu	0.5	0.2	0.02	0.01
206,207,208 Pb	0.6	0.3	0.05	0.02
55 Mn	0.1	0.05	0.02	0.04
202 Hg	n.a.	n.a.	n.a	0.2
98 Mo	0.3	0.1	0.01	0.01
60 Ni	0.5	0.2	0.06	0.03
82 Se	7.9	3.2	2.1	0.5
107 Ag	0.1	0.05	0.005	0.005
205 Tl	0.3	0.1	0.02	0.01
232 Th	0.1	0.05	0.02	0.01
238 U	0.1	0.05	0.01	0.01
51 V	2.5	1.0	0.9	0.05
66 Zn	1.8	0.7	0.1	0.2

[1]Data acquisition mode given in Table 6. Total recoverable MDL concentrations are computed for original matrix with allowance for sample dilution during preparation. Listed MDLs for solids calculated from determined aqueous MDLs.

[2]MDLs determined using state-of-the-art instrumentation (1994). Data for ^{75}As, ^{77}Se, and ^{82}Se were acquired using a dwell time of 4.096 seconds with 1500 area count per seconds ^{83}Kr present in argon supply. All other data were acquired using a dwell time of 1.024 seconds per AMU monitored.

[3]MDLs were determined from analysis of seven undigested aqueous sample aliquots.

n.a. - Not applicable. Total recoverable digestion not suitable for organo-mercury compounds.

APPENDIX A TO PART 136
METHODS FOR ORGANIC CHEMICAL ANALYSIS OF MUNICIPAL AND INDUSTRIAL WASTEWATER

METHOD 603—ACROLEIN AND ACRYLONITRILE

1. Scope and Application

1.1 This method covers the determination of acrolein and acrylonitrile. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
Acrolein	34210	107-02-8
Acrylonitrile	34215	107-13-1

1.2 This is a purge and trap gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR Part 136.1. When this method is used to analyze unfamiliar samples for either or both of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 624 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for the parameters listed above, if used with the purge and trap conditions described in this method.

1.3 The method detection limit (MDL, defined in Section 12.1)[1] for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 Any modification of this method, beyond those expressly permitted, shall be considered as a major modification subject to application and approval of alternate test procedures under 40 CFR Parts 136.4 and 136.5.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the operation of a purge and trap system and a gas chromatograph and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 An inert gas is bubbled through a 5 mL water sample contained in a heated purging chamber. Acrolein and acrylonitrile are transferred from the aqueous phase to the vapor phase. The vapor is swept through a sorbent trap where the analytes are trapped. After the purge is completed, the trap is heated and backflushed with the inert gas to desorb the compound onto a gas chromatographic column. The gas chromatograph is

Table 1—Chromatographic Conditions and Method Detection Limits

Parameter	Retention time (min)		Method detection limit (μg/L)
	Column 1	Column 2	
Acrolein	10.6	8.2	0.7
Acrylonitrile	12.7	9.8	0.5

Column 1 conditions: Porapak-QS (80/100 mesh) packed in a 10 ft x 2 mm ID glass or stainless steel column with helium carrier gas at 30 mL/min flow rate. Column temperature held isothermal at 110°C for 1.5 minutes (during desorption), then heated as rapidly as possible to 150°C and held for 20 minutes; column bakeout at 190°C for 10 minutes.[9]

Column 2 conditions: Chromosorb 101 (60/80 mesh) packed in a 6 ft x 0.1 in ID glass or stainless steel column with helium carrier gas at 40 mL/min flow rate. Column temperature held isothermal at 80°C for four minutes, then programmed at 50°C/min to 120°C and held for 12 minutes.

Table 2—Single Laboratory Accuracy and Precision—Method 603

Parameter	Sample matrix	Spike conc. (μg/L)	Average recovery (μg/L)	Standard deviation (μg/L)	Average percent recovery
Acrolein	RW	5.0	5.2	0.2	104
	RW	50.0	51.4	0.7	103
	POTW	5.0	4.0	0.2	80
	POTW	50.0	44.4	0.8	89
	IW	5.0	0.1	0.1	2
	IW	100.0	9.3	1.1	9
Acrylonitrile	RW	5.0	4.2	0.2	84
	RW	50.0	51.4	1.5	103
	POTW	20.0	20.1	0.8	100
	POTW	100.0	101.3	1.5	101
	IW	10.0	9.1	0.8	91
	IW	100.0	104.0	3.2	104

RW = Reagent water.
POTW = Prechlorination secondary effluent from a municipal sewage treatment plant.
IW = Industrial wastewater containing an unidentified acrolein reactant.

APPENDIX A TO PART 136
METHODS FOR ORGANIC CHEMICAL ANALYSIS OF MUNICIPAL AND INDUSTRIAL WASTEWATER

METHOD 608—ORGANOCHLORINE PESTICIDES AND PCBS

1. Scope and Application

1.1 This method covers the determination of certain organochlorine pesticides and PCBs. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Aldrin	39330	309-00-2
α-BHC	39337	319-84-6
β-BHC	39338	319-85-7
δ-BHC	34259	319-86-8
γ-BHC	39340	58-89-9
Chlordane	39350	57-74-9
4,4'-DDD	39310	72-54-8
4,4'-DDE	39320	72-55-9
4,4'-DDT	39300	50-29-3
Dieldrin	39380	60-57-1
Endosulfan I	34361	959-98-8
Endosulfan II	34356	33212-65-9
Endosulfan sulfate	34351	1031-07-8
Endrin	39390	72-20-8
Endrin aldehyde	34366	7421-93-4
Heptachlor	39410	76-44-8
Heptachlor epoxide	39420	1024-57-3
Toxaphene	39400	8001-35-2
PCB-1016.	34671	12674-11-2
PCB-1221	39488	1104-28-2
PCB-1232	39492	11141-16-5
PCB-1242	39496	53469-21-9
PCB-1248	39500	12672-29-6
PCB-1254	39504	11097-69-1
PCB-1260	39508	11096-82-5

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR Part 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract produced by this method.

1.3 The method detection limit (MDL, defined in Section 14.1)[1] for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in Methods 606, 609, 611, and 612. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, to apply appropriate cleanup procedures. The analyst is allowed the latitude, under Section 12, to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters.

1.5 Any modification of this method, beyond those expressly permitted, shall be considered as a major modification subject to application and approval of alternate test procedures under 40 CFR Parts 136.4 and 136.5.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of a gas chromatograph and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. The extract is separated by gas chromatography and the parameters are then measured with an electron capture detector.[2]

2.2 The method provides a Florisil column cleanup procedure and an elemental sulfur removal procedure to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.1.3.

3.1.1 Glassware must be scrupulously cleaned.[3] Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by detergent washing with hot water, and rinses with tap water and distilled water. The glassware should then be drained dry, and heated in a muffle furnace at 400°C for 15-30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Thorough rinsing with such solvents usually eliminates PCB interference. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent

Table 1—Chromatographic Conditions and Method Detection Limits

Parameter	Retention time (min)		Method detection limit (µg/L)
	Column 1	Column 2	
α-BHC	1.35	1.82	0.003
γ-BHC	1.70	2.13	0.004
β-BHC	1.90	1.97	0.006
Heptachlor	2.00	3.35	0.003
δ-BHC	2.15	2.20	0.009
Aldrin	2.40	4.10	0.004
Heptachlor epoxide	3.50	5.00	0.083
Endosulfan I	4.50	6.20	0.014
4,4'-DDE	5.13	7.15	0.004
Dieldrin	5.45	7.23	0.002
Endrin	6.55	8.10	0.006
4,4'-DDD	7.83	9.08	0.011
Endosulfan II	8.00	8.28	0.004
4,4'-DDT	9.40	11.75	0.012
Endrin aldehyde	11.82	9.30	0.023
Endosulfan sulfate	14.22	10.70	0.066
Chlordane	mr	mr	0.014
Toxaphene	mr	mr	0.240
PCB-1016	mr	mr	nd
PCB-1221	mr	mr	nd
PCB-1232	mt	mr	nd
PCB-1242	mr	mr	0.065
PCB-1248	mr	mr	nd
PCB-1254	mr	mr	nd
PCB-1260	mr	mr	nd

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95% SP-2401 packed in a 1.8 m long x 4 mm ID glass column with 5% methane/95% argon carrier gas at 60 mL/min. flow rate. Column temperature held isothermal at 200°C, except for PCB-1016 through PCB-1248, should be measured at 160°C.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% OV-one packed in a 1.8 m long x 4 mm ID glass column with 5% methane/95% argon carrier gas at 60 mL/min. flow rate. Column temperature held isothermal at 200°C for the pesticides; at 140°C for PCB-1221 and 1232; and at 170°C for PCB-1016 and 1242-1268.

mr = Multiple peak response. See Figures 2-10.
nd = Not determined.

APPENDIX A TO PART 136
METHODS FOR ORGANIC CHEMICAL ANALYSIS OF MUNICIPAL AND INDUSTRIAL WASTEWATER

METHOD 613—2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

1. Scope and Application

1.1 This method covers the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). The following parameter may be determined by this method:

Parameter	STORET No.	CAS No.
2,3,7,8-TCDD	34675	1746-01-6

1.2 This is a gas chromatographic/mass spectrometer (GC/MS) method applicable to the determination of 2,3,7,8-TCDD in municipal and industrial discharges as provided under 40 CFR Part 136.1. Method 625 may be used to screen samples for 2,3,7,8-TCDD. When the screening test is positive, the final qualitative confirmation and quantification must be made using Method 613.

1.3 The method detection limit (MDL, defined in Section 14.1)[1] for 2,3,7,8-TCDD is listed in Table 1. The MDL for a specific wastewater may be different from that listed, depending upon the nature of interferences in the sample matrix.

1.4 Because of the extreme toxicity of this compound, the analyst must prevent exposure to himself, of to others, by materials knows or believed to contain 2,3,7,8-TCDD. Section 4 of this method contains guidelines and protocols that serve as minimum safe-handling standards in a limited-access laboratory.

1.5 Any modification of this method, beyond those expressly permitted, shall be considered as a major modification subject to application and approval of alternate test procedures under 40 CFR Parts 136.4 and 136.5.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of a gas chromatograph/mass spectrometer and in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1 L, is spiked with an internal standard of labeled 2,3,7,8-TCDD and extracted with methylene chloride using a separatory funnel. The methylene chloride extract is exchanged to hexane during concentration to a volume of 1.0 mL or less. The extract is then analyzed by capillary column GC/MS to separate and measure 2,3,7,8-TCDD.2,3

13. "Methods, 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

14. Wong, A.S. et al. "The Determination of 2,3,7,8-TCDD in Industrial and Municipal Wastewaters, Method 613, Part 1-Development and Detection Limits," G. Choudhay, L. Keith, and C. Ruppe, ed., Butterworth Inc., (1983).

15. "EPA Method Study 26, Method 613: 2,3,7,8-Tetrachlorodibenzo-p-dioxin," EPA 600/4-84-037, National Technical Information Service, PB84-188879, Springfield, Virginia 22161, May1984.

Table 1—Chromatographic Conditions and Method Detection Limit

Parameter	Retension time (min)	Method detection limit (µg/L)
2,3,7,8-TCDD	13.1	0.002

Column conditions: SP-2330 coated on a 60 m long x 0.25 mm ID glass column with hydrogen carrier gas at 40 cm/sec. linear velocity, splitless injection using tetradecane. Column temperature held isothermal at 200°C for one minute, then programmed at 8°C/min. to 250°C and held. Use of helium carrier gas will approximately double the retention time.

Table 2—QC Acceptance Criteria—Method 613

Parameter	Test Conc. (µg/L)	Limit for s (µg/L)	Range for $\bar{X}$ (µg/L)	Range for P, P_s (%)
2,3,7,8-TCDD	0.100	0.0276	0.0523-0.1226	45-129

s = Standard deviation of four recovery measurements, in µ/L (Section 8.2.4).
$\bar{X}$ = Average recovery for four recovery measurements, in µ/L (Section 8.2.4).
P, P_s = Percent recovery measured (Section 8.3.2, Section 8.4.2).

NOTE: These criteria are based directly upon the method performance data in Table 3. Where necessary, the limits for recovery have been broadened to assure applicability of the limits to concentrations below those used to develop Table 3.

APPENDIX A TO PART 136
METHODS FOR ORGANIC CHEMICAL ANALYSIS OF MUNICIPAL AND INDUSTRIAL WASTEWATER

METHOD 624—PURGEABLES

1. Scope and Application

1.1 This method covers the determination of a number of purgeable organics. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
Benzene	34030	71-43-2
Bromodichloromethane	32101	75-27-4
Bromoform	32104	75-25-2
Bromomethane	34413	74-83-9
Carbon tetrachloride	32102	56-23-5
Chlorobenzene	34301	108-90-7
Chloroethane	34311	75-00-3
2-Chloroethylvinyl ether	34576	110-75-8
Chloroform	32106	67-66-3
Chloromethane	34418	74-87-3
Dibromochloromethane	32105	124-48-1
1,2-Dichlorobenzene	34536	95-50-1
1,3-Dichlorobenzene	34566	541-73-1
1,4-Dichlorobenzene	34571	106-46-7
1,1-Dichloroethane	34496	75-34-3
1,2-Dichloroethane	34531	107-06-2
1,1-Dichloroethane	34501	75-35-4
trans-1,2-Dichloroethene	34546	156-60-5
1,2-Dichloropropane	34541	78-87-5
cis-1,3-Dichloropropene	34704	10061-01-5
trans-1,3-Dichloropropene	34699	10061-02-6
Ethyl benzene	34371	100-41-4
Methylene chloride	34423	75-09-2
1,1,2,2-Tetrachloroethane	34516	79-34-5
Tetrachloroethene	34475	127-18-4
Toluene	34010	108-88-3
1,1,1-Trichloroethene	34506	71-55-6
1,1,2-Trichloroethene	34511	79-00-5
Trichloroethane	39180	79-01-6
Trichlorofluoromethane	34488	75-69-4
Vinyl chloride	39175	75-01-4

1.2 The method may be extended to screen samples for acrolein (STORET No. 34210, CAS No. 107-02-8) and acrylonitrile (STORET No. 34215, CAS No. 107-13-1), however, the preferred method for these two compounds is Method 603.

1.3 This is a purge and trap gas chromatographic/mass spectrometer (GC/MS) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR Part 136.1.

1.4 The method detection limit (MDL, defined in Section 14.1)[1] for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 Any modification to this method, beyond those expressly permitted, shall be considered as a major modification subject to application and approval of alternate test procedures under 40 CFR Parts 136.4 and 136.5. Depending upon the nature of the modification and the extent of intended use, the applicant may be required to demonstrate that the modifications will produce equivalent results when applied to relevant wastewaters.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the operation of a purge and trap system and a gas chromatograph/mass spectrometer and in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 An inert gas is bubbled through a 5 mL water sample contained in a specially-designed purging chamber at ambient temperature. The purgeables are efficiently transferred from the aqueous phase to the vapor phase. The vapor is swept through a sorbent trap where the purgeables are trapped. After purging is completed, the trap is heated and backflushed with the inert gas to desorb the purgeables onto a gas chromatographic column. The gas chromatograph is temperature programmed to separate the purgeables which are then detected with a mass spectrometer.[2,3]

3. Interferences

3.1 Impurities in the purge gas, organic compounds outgassing from the plumbing ahead of the trap, and solvent vapors in the laboratory account for the majority of contamination problems. The analytical system must be demonstrated to be free from contamination under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.1.3. The use of non-Teflon plastic tubing, non-Teflon thread sealants, or flow controllers with rubber components in the purge and trap system should be avoided.

3.2 Samples can be contaminated by diffusion of volatile organics (particularly fluorocarbons and methylene chloride) through the septum seal into the sample during shipment and storage. A field reagent blank prepared from reagent water and carried through the sampling and handling protocol can serve as a check on such contamination.

3.3 Contamination by carry-over can occur whenever high level and low level samples are sequentially analyzed. To reduce carry-over, the purging device and sample syringe must be rinsed with reagent water between sample analyses. Whenever an

Table 1—Chromatographic Conditions and Method Detection Limits

Parameter	Retention time (min.)	Method detection limit(µ/L)
Chloromethane	2.3	nd
Bromomethane	3.1	nd
Vinyl chloride	3.8	nd
Chloroethane	4.6	nd
Methylene chloride	6.4	2.8
Trichlorofluoromethane	8.3	nd
1,1-Dichloroethene	9.0	2.8
1,1-Dichloroethane	10.1	4.7
trans-1,2-Dichloroethene	10.8	1.6
Chloroform	11.4	1.6
1,2-Dichloroethane	12.1	2.8
1,1,1-Trichloroethane	13.4	3.8
Carbon tetrachloride	13.7	2.8
Bromodichloromethane	14.3	2.2
1,2-Dichloroproane	15.7	6.0
cis-1,3-Dichloropropene	15.9	5.0
Trichloroethene	16.5	1.9
Benzene	17.0	4.4
Dibromochloromethane	17.1	3.1
1,1,2-Trichloroethane	17.2	5.0
trans-1,3-Dichloropropene	17.2	nd
2-Chloroethylvinlyl ether	18.6	nd
Bromoform	19.8	4.7
1,1,2,2-Tetrachloroethane	22.1	6.9
Tetrachloroethene	22.2	4.1
Toluene	23.5	6.0
Chlorobenzene	24.6	6.0
Ethyl benzene	26.4	7.2
1,3-Dichlorobenzene	33.9	nd
1,2-Dichlorobenzene	35.0	nd
1,4-Dichlorobenzene	35.4	nd

Column conditions: Carbopak B (60/80 mesh) coated with 1% SP-1000 packed in a 6 ft by 0.1 in. ID glass column with helium carrier gas at 30 mL/min. flow rate. Column temperature held at 45°C for three minutes, then programmed at 8°C/min. to 220°C and held for 15 minutes.

nd = Not determined.

APPENDIX A TO PART 136
METHODS FOR ORGANIC CHEMICAL ANALYSIS OF MUNICIPAL AND INDUSTRIAL WASTEWATER

METHOD 625—BASE/NEUTRALS AND ACIDS

1. Scope and Application

1.1 This method covers the determination of a number of organic compounds that are partitioned into an organic solvent and are amenable to gas chromatography. The parameters listed in Tables 1 and 2 may be qualitatively and quantitatively determined using this method.

1.2 The method may be extended to include the parameters listed in Table 3. Benzidine can be subject to oxidative losses during solvent concentration. Under the alkaline conditions of the extraction step, α-BHC, γ-BHC, endosulfan I and II, and endrin are subject to decomposition. Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution, and photochemical decomposition. N-nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described. N-nitrosodiphenylamine decomposes in the gas chromatographic inlet and cannot be separated from diphenylamine. The preferred method for each of these parameters is listed in Table 3.

1.3 This is a gas chromatographic/mass spectrometry (GC/MS) method[2,14] applicable to the determination of the compounds listed in Tables 1, 2, and 3 in municipal and industrial discharges as provided under 40 CFR Part 136.1.

1.4 The method detection limit (MDL, defined in Section 16.1)[1] for each parameter is listed in Tables 4 and 5. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 Any modification to this method, beyond those expressly permitted, shall be considered as a major modification subject to application and approval of alternate test procedures under 40 CFR Parts 136.4 and 136.5. Depending upon the nature of the modification and the extent of intended use, the applicant may be required to demonstrate that the modifications will produce equivalent results when applied to relevant wastewaters.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of a gas chromatograph/mass spectrometer and in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1 L, is serially extracted with methylene chloride at a pH greater than 11 and again at a pH less than 2 using a separatory funnel or a continuous extractor.[2] The methylene chloride extract is

Table 2--Acid Extractables

Parameter	STORET No.	CAS No.
4-Chloro-3-methylphenol	34452	59-50-7
2-Chlorophenol	34586	95-57-8
2,4-Dichlorophenol	34601	120-83-2
2,4-Dimethylphenol	34606	105-67-9
2,4-Dinitrophenol	34616	51-28-5
2-Methyl-4,6-dinitrophenol	34657	534-52-1
2-Nitrophenol	34591	88-75-5
4-Nitrophenol	34646	100-02-7
Pentachlorophenol	39032	87-86-5
Phenol	34694	108-95-2
2,4,6-Trichlorophenol	34621	88-06-2

Table 3—Additional Extractable Parameters[a]

Parameter	STORET No.	CAS No.	Method
Benzidine	39120	92-87-5	605
β-BHC	39337	319-84-6	608
δ-BHC	39340	58-89-8	608
Endosulfan I	34361	959-98-8	608
Endosulfan II	34356	33213-65-9	608
Endrin	39390	72-20-8	608
Hexachlorocylopentadiene	34386	77-47-4	612
N-Nitrosodimethylamine	34438	62-75-9	607
N-Nitrosodiphenylamine	34433	86-30-6	607

[a]See Section 1.2.

Table 4—Chromatographic Conditions, Method Detection Limits, and Characteristic Masses for Base/Neutral Extractables

Parameter	Retention time (min)	Method detection limit (µg/L)	Characteristic masses					
			Electron impact			Chemical ionization		
			Primary	Secondary	Secondary	Methane	Methane	Methane
1,3-Dichlorobenzene	7.4	1.9	146	148	113	146	148	150
1,4-Dichlorobenzene	7.8	4.4	146	148	113	146	148	150
Hexachloroethane	8.4	1.6	117	201	199	199	201	203
Bis(2-chloroethyl) ether[a]	8.4	5.7	93	63	95	63	107	109
1,2-Dichlorobenzene	8.4	1.9	146	148	113	146	148	150
Bis(2-chloroisopropyl) ether[a]	9.3	5.7	45	77	79	77	135	137
N-Nitrosodi-n-propylamine	.....	.....	130	42	101	.....	.....	.....
Nitrobenzene	11.1	1.9	77	123	65	124	152	164

Table 4—Chromatographic Conditions, Method Detection Limits, and Characteristic Masses for Base/Neutral Extractables

Parameter	Retention time (min)	Method detection limit (µg/L)	Characteristic masses					
			Electron impact			Chemical ionization		
			Primary	Secondary	Secondary	Methane	Methane	Methane
Hexachlorobutadiene	11.4	0.9	225	223	227	223	225	227
1,2,4-Trichlorobenzene	11.6	1.9	180	182	145	181	183	209
Isophorone	11.9	2.2	82	95	138	139	167	178
Naphthalene	12.1	1.6	128	129	127	129	157	169
Bis(2-chloroethoxy) methane	12.2	5.3	93	95	123	65	107	137
Hexachlorocyclopentadiene[a]	13.9	.....	237	235	272	235	237	239
2-Chloronaphthalene	15.9	1.9	162	164	127	163	191	203
Acenaphthylene	17.4	3.5	152	151	153	152	153	181
Acenaphthene	17.8	1.9	154	153	152	154	155	183
Dimethyl phthalate	18.3	1.6	163	194	164	151	163	164
2,6-Dinitrotoluene	18.7	1.9	165	89	121	183	211	223
Fluorene	19.5	1.9	166	165	167	166	167	195
4-Chlorophenyl phenyl ether	19.5	4.2	204	206	141	.....	.....	.....
2,4-Dinitrotoluene	19.8	5.7	165	63	182	183	211	223
Diethyl phthalate	20.1	1.9	149	177	150	177	223	251
N-Nitrosodiphenylamine[b]	20.5	1.9	169	168	167	169	170	198
Hexachlorobenzene	21.0	1.9	284	142	249	284	286	288
β-BHC[b]	21.1	.....	183	181	109	.....	.....	.....
4-Bromophenyl phenyl ether	21.2	1.9	248	250	141	249	251	277
δ-BHC[b]	22.4	.....	183	181	109	.....	.....	.....
Phenanthrene	22.8	5.4	178	179	176	178	179	207
Anthracene	22.8	1.9	178	179	176	178	179	207
β-BHC	23.4	4.2	181	183	109	.....	.....	.....
Heptachlor	23.4	1.9	100	272	274	.....	.....	.....
δ-BHC	23.7	3.1	183	109	181	.....	.....	.....
Aldrin	24.0	1.9	66	263	220	.....	.....	.....
Dibutyl phthalate	24.7	2.5	149	150	104	149	205	279
Heptachlor epoxide	25.6	2.2	353	355	351	.....	.....	.....
Endosulfan I[b]	26.4	.....	237	339	341	.....	.....	.....
Fluoranthene	26.5	2.2	202	101	100	203	231	243
Dieldrin	27.2	2.5	79	263	279	.....	.....	.....
4,4'-DDE	27.2	5.6	246	248	176	.....	.....	.....
Pyrene	27.3	1.9	202	101	100	203	231	243
Endrin[b]	27.9	.....	81	263	82	.....	.....	.....
Endosulfan II[b]	28.6	.....	237	339	341	.....	.....	.....
4,4'-DDD	28.6	2.8	235	237	165	.....	.....	.....
Benzidine[b]	28.8	44	184	92	185	185	213	225
4,4'-DDT	29.3	4.7	235	237	165	.....	.....	.....

Table 4—Chromatographic Conditions, Method Detection Limits, and Characteristic Masses for Base/Neutral Extractables

Parameter	Retention time (min)	Method detection limit (µg/L)	Characteristic masses					
			Electron impact			Chemical ionization		
			Primary	Secondary	Secondary	Methane	Methane	Methane
Endosulfan sulfate	29.8	5.6	272	387	422	.....	.....	.....
Endrin aldehyde	.....	.....	67	345	250	.....	.....	.....
Butyl benzyl phthalate	29.9	2.5	149	91	206	149	299	327
Bis(2-ethylhexyl) phthalate	30.6	2.5	149	167	279	149	.....	.....
Chrysene	31.5	2.5	228	226	229	228	229	257
Benzo(a)anthracene	31.5	7.8	228	229	226	228	229	257
3,3'-Dichlorobenzidine	32.2	16.5	252	254	126	.....	.....	.....
Di-n-octyl phthalate	32.5	2.5	149	.....	.....	.....	.....	.....
Benzo(b)fluoranthene	34.9	4.8	252	253	125	252	253	281
Benzo(k)fluoranthene	34.9	2.5	252	253	125	252	253	281
Benzo(a)pyrene	36.4	2.5	252	253	125	252	253	281
Indeno(1,2,3-cd) pyrene	42.7	3.7	276	138	277	276	277	305
Dibenzo(a,h) anthracene	43.2	2.5	278	139	279	278	279	307
Benzo(ghi)perylene	45.1	4.1	276	138	277	276	277	305
N-Nitrosodimethyl-amine[b]	.....	.....	42	74	44	.....	.....	.....
Chlordane[c]	19-30	.....	373	375	377	.....	.....	.....
Toxaphene[c]	25-34	.....	159	231	233	.....	.....	.....
PCB 1016[c]	18-30	.....	224	260	294	.....	.....	.....
PCB 1221[c]	15-30	30	190	224	260	.....	.....	.....
PCB 1232[c]	15-32	.....	190	224	260	.....	.....	.....
PCB 1242[c]	15-32	.....	224	260	294	.....	.....	.....
PCB 1248[c]	12-34	.....	294	330	262	.....	.....	.....
PCB 1254[c]	22-34	36	294	330	362	.....	.....	.....
PCB 1260[c]	23-32	.....	330	362	394	.....	.....	.....

[a]The proper chemical name is 2,2'-bisoxy(1-chloropropane).

[b]See Section 1.2.

[c]These compounds are mixtures of various isomers (See Figures 2 through 12). Column conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a 1.8 m long x 2 mm ID glass column with helium carrier gas at 30 mL/min. flow rate. Column temperature held isothermal at 50°C for four minutes, then programmed at 8°C/min. to 270°C and held for 30 minutes.

Table 5—Chromatographic Conditions, Method Detection Limits, and Characteristic Masses for Acid Extractables

Parameter	Retention time (min)	Method detection limit (µg/L)	Characteristic masses					
			Electron impact			Chemical ionization		
			Primary	Secondary	Secondary	Methane	Methane	Methane
2-Chlorophenol	5.9	3.3	128	64	130	129	131	157
2-Nitrophenol	6.5	3.6	139	65	109	140	168	122
Phenol	8.0	1.5	94	65	66	95	123	135
2,4-Dimethylphenol	9.4	2.7	122	107	121	123	151	163
2,4-Dichlorophenol	9.8	2.7	162	164	98	163	165	167
2,4,6-Trichlorophenol	11.8	2.7	196	198	200	197	199	201
4-Chloro-3-methylphenol	13.2	3.0	142	107	144	143	171	183
2,4-Dinitrophenol	15.9	42	184	63	154	185	213	225
2-Methyl-4,6-dinitrophenol	16.2	24	198	182	77	199	227	239
Pentachlorophenol	17.5	3.6	266	264	268	267	265	269
4-Nitrophenol	20.3	2.4	65	139	109	140	168	122

Column conditions: Supelcoport (100/120 mesh) coated with 1% SP-1240DA packed in a 1.8 m long x 2mm ID glass column with helium carrier gas at 30 mL/min. flow rate. Column temperature held isothermal at 70°C for two mintues then programmed at 8°C/min. to 200°C.

Table 6—QC Acceptance Criteria—Method 625

Parameter	Test conclusion (µg/L)	Limits for s (µg/L)	Range for $\bar{X}$ (µg/L)	Range for P, P_s (Percent)
Acenaphthene	100	27.6	60.1-132.3	47-145
Acenaphthylene	100	40.2	53.5-126.0	33-145
Aldrin	100	39.0	7.2-152.2	D-166
Anthracene	100	32.0	43.4-118.0	27-133
Benzo(a)anthracene	100	27.6	41.8-133.0	33-143
Benzo(b)fluoranthene	100	38.8	42.0-140.4	24-159
Benzo(k)fluoranthene	100	32.3	25.2-145.7	11-162
Benzo(a)pyrene	100	39.0	31.7-148.0	17-163
Benzo(ghi)perylene	100	58.9	D-195.0	D-219
Benzyl butyl phthalate	100	23.4	D-139.9	D-152
β-BHC	100	31.5	41.5-130.6	24-149
δ-BHC	100	21.6	D-100.0	D-110
Bis(2-chloroethyl)ether	100	55.0	42.9-126.0	12-158
Bis(2-chloroethoxy)methane	100	34.5	49.2-164.7	33-184
Bis(2-chloroisopropyl)ether[a]	100	46.3	62.8-138.6	36-166
Bis(2-ethylhexyl) phthalate	100	41.1	28.9-136.8	8-158
4-Bromophenyl phenyl ether	100	23.0	64.9-114.4	53-127
2-Chloronaphthalene	100	13.0	64.5-113.5	60-118
4-Chlorophenyl phenyl ether	100	33.4	38.4-144.7	25-158
Chrysene	100	48.3	44.1-139.9	17-168