

Use of tissue and sediment-based threshold concentrations of polychlorinated biphenyls (PCBs) to protect juvenile salmonids listed under the US Endangered Species Act

JAMES P. MEADOR*, TRACY K. COLLIER and JOHN E. STEIN

Environmental Conservation Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA, 2725 Montlake Blvd. East, Seattle, WA 98112, USA

ABSTRACT

1. Under the Endangered Species Act, the National Marine Fisheries Service has authority to protect listed species from any adverse actions that may jeopardize the population's ability to recover and increase to sustainable levels. Listed salmon species in the northwest United States are known to travel through urban areas in their migration from river to ocean. Species such as the chinook salmon (*Oncorhynchus tshawytscha*) often spend several weeks in these urban estuaries where they can be highly exposed to urban-related contaminants that reside in the sediments and accumulate in their prey species. The concern is that these contaminants are bioaccumulated to levels that may impact the ability of individual salmon to grow and mature normally. This paper provides a framework for determining the tissue and sediment concentrations of polychlorinated biphenyls (PCBs) that are likely protective against adverse effects in listed salmonid species.

2. The relevant ecotoxicological literature was examined and 15 studies were selected that met the pre-established criteria outlined here. For each study, the lowest tissue concentration (residue) of total PCBs associated with a biological response was selected. The tissue concentration associated with the 10th percentile of these 15 studies was chosen to represent the residue effect threshold (RET) above which wild juvenile salmonids would be expected to exhibit adverse sublethal effects from accumulated PCBs. This value ($2.4 \mu\text{g PCBs g}^{-1}$ lipid) is expressed in terms of the lipid-normalized concentration because of the large effect lipid can have on the expressed toxicity and the substantial variability in lipid content observed in salmonids over their life cycle.

3. A sediment concentration that is expected to produce the RET was then determined using the biota-sediment accumulation factor approach. The sediment effect threshold, which varies with the total organic carbon content in sediment, is the level above which adverse effects may be expected in juvenile salmonids due to accumulation of PCBs from environmental exposure. Bioaccumulation of PCBs was examined in one river system as a model for determining an appropriate bioaccumulation factor for wild juvenile chinook salmon.

4. Evaluation of exposure to potentially deleterious concentrations of PCBs based on tissue residues is the preferred approach; however, the sediment effect threshold may also be used in cases where bioaccumulation has been characterized in an estuary. The threshold values presented here are intended as interim guidelines that should be modified as more data become available. Additionally, because of the uncertainty around many of the factors and assumptions that comprise the single

*Correspondence to: Dr. James P. Meador, Environmental Conservation Division, Northwest Fisheries Science Center, NMFS-NOAA, 2725 Montlake Blvd. East, Seattle WA 98112, USA. E-mail: james.meador@noaa.gov

threshold effect values, it is recommended that future studies be employed to help determine a range of acceptable values that would afford protection under various environmental and biological conditions.

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INTRODUCTION AND BACKGROUND

The goal of this paper is to present the methods used to determine tissue and sediment threshold concentrations of polychlorinated biphenyls (PCBs) in juvenile salmonids. These threshold concentrations are levels that the National Marine Fisheries Service (NMFS) considers protective against adverse effects and promotes recovery of the listed population. The approach was to examine the literature concerning PCB toxicity in salmonids (not including early life stages) whereby a tissue residue could be associated with a biological response. Once the residue effect threshold (RET) was established, the properties of bioaccumulation were used to predict a sediment concentration that would result in the RET. Even though the RET is preferred for assessing potential effects, the determination of a sediment threshold may be desirable under certain circumstances. This analysis focused on juvenile salmonids because in the Pacific Northwest, the highest exposure to contaminants occurs in urban estuaries where migrating salmon complete their physiological transition from freshwater to marine water.

Endangered Species Act

Species listed under the Endangered Species Act (ESA) are afforded protection from several types of adverse actions. Sections 4(d) and 9 of the ESA prohibit 'take' of a listed species, which is defined as harming, harassing, pursuing, hunting, shooting, wounding, killing, trapping, capturing, or collecting of listed species without a specific permit or exemption. The term 'harm', which is part of the definition for take, extends the list of acts to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing essential behavioural patterns such as breeding, feeding and sheltering. The term 'harm' was recently clarified by the National Oceanic and Atmospheric Administration (NOAA) (NOAA, 1999) and several activities that may constitute a take were listed. Among the activities listed included discharging of pollutants, such as oil or toxic chemicals into a listed species' habitat, as well as contamination of other biota, such as prey, required by the listed species for these essential behavioural patterns.

Water and sediment quality guidelines are generally based on analyses of the responses exhibited by several species to a toxicant. Often the goal is to protect 95% of all species from effects that may impact population abundance (Stephan *et al.*, 1985). Consequently, these guidelines are based on the biological responses (mortality, and alterations to growth and reproduction), which are generally recognized as responses that will likely impact population dynamics. In contrast to this, protection of listed species under the ESA must consider harm to individual fish because additional losses will impede the recovery of an already severely depleted population. Because the probability of extinction is higher for ESA listed species, protection of individuals is important to ensure overall population recovery to a more stable level. While the standard sublethal responses of growth and reproductive impairment may be reasonably translated into population effects, all other sublethal responses, such as altered hormone levels, increased enzyme activity and disease susceptibility were considered in this analysis. This is primarily due to the potential effects that PCBs may have on the complex physiological processes that allow individual salmon to make the transition from a freshwater to marine mode of existence, resist disease, mature normally and successfully complete their life cycle.

Endpoint/response selection

The goal for this analysis was to develop a tissue-based threshold concentration for PCB contamination in juvenile salmonids below which sublethal effects are not expected to occur. Many biological responses have been reported for PCBs, including mortality, impaired growth and reproduction, immune dysfunction, hormonal alterations, enzyme induction, neurotoxicity, behavioural responses, disease susceptibility and mutagenicity. While some biological responses, such as mortality, growth inhibition, and reproductive impairment, would likely have measurable impacts on a population (Forbes and Calow, 1999), other endpoints, such as altered hormone levels or induced enzyme systems, may also have adverse physiological effects on salmonids thereby reducing their fitness. For example, thyroid function is associated with many physiological processes in fish metabolism. As noted by Mayer *et al.* (1977), thyroid metabolism plays a role in respiration, carbohydrate and ammonia metabolism, oxygen consumption, nervous system function and behaviour. Although it is well known that induction of the cytochrome P450 system will lead to an increase in production of mutagenic compounds, these enzymes may also be involved in altering essential steroid hormones that are required for normal physiological processes (Di Giulio *et al.*, 1995).

Impairment of these vital functions may affect a fish's ability to tolerate normal environmental fluctuations, including the physiologically demanding process of smoltification (the ability to transition from freshwater to seawater). Several physiological parameters (e.g. ATPase levels in the gill, thyroid and pituitary hormones, liver glycogen, blood glucose and lipid metabolism) change during the parr to smolt transformation in salmonids (Wedemeyer *et al.*, 1980). Alteration of any associated physiological functions may substantially reduce the chances of successful smoltification and the individual's ability to thrive and mature in the marine environment. For example, a recent eco-epidemiological study (Fairchild *et al.*, 1999) showed a strong negative association between catch of returning adult salmon and the percent of the watershed sprayed with nonylphenol (a solvent used to apply the pesticide aminocarb). The authors suggested that this historical decline in returning fish was due to nonylphenol-induced changes in the endocrine system of juvenile outmigrant salmon and possible effects on smoltification. Many studies have demonstrated that PCBs can affect the thyroid hormones important for smoltification in salmon (Mayer *et al.*, 1977; Folmar *et al.*, 1982), which supports their ecological relevance and inclusion in this analysis.

Toxicity equivalent factors (TEFs)

In recent work it has been shown that some PCB congeners are much more toxic than others, which is primarily a function of the position of the chlorine atoms and their ability to interact with the aryl hydrocarbon (Ah) receptor. The most toxic PCBs are the non- and mono-ortho substituted congeners, which tend to be planar compounds. Some of these responses listed above, such as developmental and reproductive abnormalities, enzyme induction, and immunosuppression, can occur at extremely low concentrations and are likely caused by 'dioxin-like' PCB congeners (planar congeners). These planar congeners can occur in the Aroclor mixtures, but usually at low concentrations. The responses caused by the non-planar congeners ('non-dioxin-like') are likely due to different modes of action and include neurotoxicity, hypothyroidism, carcinogenicity, behavioural alteration and endocrine disruption (Giesy and Kannan, 1998).

The toxicity equivalent factor (TEF) approach has been used to determine the relative toxicity of the planar PCB congeners as a fraction of the toxicity elicited by 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). Tissue concentrations of PCB congeners are multiplied by the TEF to generate a toxicity equivalent (TEQ) concentration in terms of its 'dioxin-like' potency. These TEQs are then summed to generate a total TEQ concentration for the sample that can be compared to dioxin toxicity results. Ideally, the TEFs should be species- and endpoint-specific because of the observed variability (Giesy and Kannan, 1998). The TEF approach is not applicable for those 'non-dioxin-like' biological responses caused by the non-planar PCB congeners, primarily due to the different modes of action.

Most TEFs have been developed for mammals and birds and only very recently for fish (Walker and Peterson, 1991). The TEFs for fish are somewhat limited because they apply only to early life stage mortality in salmonids and enzyme induction (Giesy and Kannan, 1998). There are no TEFs for biological effects occurring beyond the embryo/alevin state. Effects of PCBs to early life stages were not considered in this analysis, primarily due to the lower risk of PCB exposure for fish in upstream areas. Because the available relevant information on PCB responses in salmonids is based on total PCB concentrations and because this study focused on juvenile salmon migrating through urban estuaries, TEFs could not be considered in assessing PCB exposure and effects. If such congener specific toxicity information becomes available for biological responses relevant for salmonid life stages beyond the embryo, then this information should be incorporated into future assessments. For example, a recent study demonstrated a significant increase in mortality for adult rainbow trout exhibiting a tissue concentration (fillet) of TCDD of only 0.44 pg g^{-1} wet wt. (Jones *et al.*, 2001). Future work linking this mortality response caused by TCDD and dioxin-like PCB congeners may be important for determining impacts to salmonids.

Tissue residue and biota–sediment accumulation factor (BSAF) approaches

One way to assess adverse effects in aquatic organisms is to relate a biological response to an exposure concentration (e.g. water, food or sediment). These data would then be used to generate an effect concentration based on the exposure media. For example, an LC50 may be generated indicating that 50% of the individuals would be expected to die when exposed to a given water concentration. Another method for assessing impacts is to relate adverse biological effects with tissue concentrations of toxicants. This method is attractive because it reduces the variability inherent in linking biological responses to exposure concentrations. First, a tissue residue deemed to be protective for a species (e.g. LOER or NOER; lowest or no observed effect tissue residue), is determined from several controlled laboratory studies for a given toxicant. With this information, LOERs for several species can be compared to determine a RET that would protect all species for a given endpoint (e.g. growth, reproduction or mortality). In some cases there are insufficient data to generate an endpoint-specific residue effect threshold or the goal is to protect one species or group of species against a range of adverse biological effects (e.g. this study). For these situations, one approach for assuring protection would be to combine all endpoints for a given species or family (e.g. salmonids) and set the RET equal to a low value (e.g. 10th percentile of all studies).

Sediment concentrations are often the focus for determining if a site is contaminated, and sediment quality guidelines or criteria are promulgated based on expected bioaccumulation and toxic effects resulting from exposure to sediment-associated toxicants. Sediment concentrations are preferred over water or food exposure concentrations because they are less variable spatially and temporally. Concentrations of contaminants in sediment are used as a surrogate for characterizing the exposure of aquatic species to these compounds found in water and the food that they ingest because concentrations of neutral organic contaminants, such as PCBs, found in water and prey items are expected to be proportional to that found in sediment (Di Toro *et al.*, 1991).

A commonly accepted method for relating tissue and sediment concentrations is by calculating a biota–sediment accumulation factor (BSAF) with the following equation:

$$\text{BSAF} = \frac{[\text{tissue}]/f_{\text{lip}}}{[\text{sediment}]/f_{\text{oc}}} \quad (1)$$

where [tissue] and [sediment] are concentrations, f_{oc} is the fraction of organic carbon (g g^{-1}) and f_{lip} is the fraction of lipid (g g^{-1}).

Several factors, such as variable uptake and elimination rates, reduced bioavailability, reduced exposure, and insufficient time for sediment–water partitioning or tissue steady state can affect bioaccumulation and

ultimately the BSAF. Because of these differences in bioaccumulation, a species- and system-specific BSAF is recommended for a more accurate representation of bioaccumulation as a function of the above factors. Additionally, the BSAF should be expressed as a function of time, if the time for exposure is known (e.g. 10 day BSAF).

It should be noted that even though the BSAF is derived from sediment concentrations, there is no implicit assumption of sediment ingestion. The BSAF value integrates exposure from all sources (prey, water and sediment ingestion) because it is assumed that the concentrations of chemicals (in this case PCBs) in the different matrices occur in predictable proportions. According to theory, the tissue concentration of the target species can be determined by using the concentration in one of the matrices to represent all others. In this case sediment concentrations are used because they are the easiest to determine, they are less variable than water or prey concentrations, they are the focus for regulatory action, and large databases already exist. This feature is especially advantageous when determining a system-specific BSAF value because it does not matter if sediment concentrations are high or low, concentrations in the different matrices are presumably related by the same proportion at all sites. Also implicit in this approach is that it does not matter if the main source of PCBs to the organism varies between ventilation of water or ingestion of sediment or prey, the sediment concentration can still be used to represent accumulation from all sources.

Once the RET is established, the following method can be employed to generate a sediment quality guideline (SQG), or in this case the sediment effect threshold (SET) for use in regulating exposure to a contaminant in a particular system. The tissue residue associated with adverse biological effects (RET) is converted to an organic-carbon-normalized sediment concentration (SET) by utilizing the species- and system-specific BSAF value. (In this case system-specific refers to a particular estuary.) The rearranged BSAF equation is

$$[\text{sed}_{\text{oc}}] = \frac{[\text{tissue}_{\text{lip}}]}{\text{BSAF}} \quad (2)$$

where sed_{oc} is the organic-carbon-normalized sediment concentration, $[\text{tissue}_{\text{lip}}]$ is the lipid-normalized tissue concentration used for protection (LOER, NOER or RET), and the BSAF is a species- and system-specific value determined with field samples. The sediment effect threshold is total organic carbon (TOC)-dependent and should be expressed in units of organic carbon ($\text{ng PCBs g}^{-1} \text{OC}$) or as a dry weight concentration ($\text{ng PCBs g}^{-1} \text{sediment}$) using the average TOC content.

Lipid as a controlling factor

It is well known that the tissue concentration of a lipophilic toxicant causing the response is directly related to the amount of lipid in an organism (Lassiter and Hallam, 1990; van Wezel *et al.*, 1995). In other words, for a given wet or dry weight tissue concentration, the higher the lipid content, the higher the resistance to the toxicant because a higher proportion of the hydrophobic compound is associated with the lipid and is not available to cause toxicity. It is also well known that salmonids exhibit variable lipid content over their life cycle with low points during the fry and smolt stages (Brett, 1995). Additionally, studies have shown that hatchery fish generally contain much higher whole-body lipids than wild fish during pre-smolt and smolt stages (Wood *et al.*, 1960; Don Larson, NMFS, pers. comm.). One recent study of wild spring chinook salmon around Yakima, Washington found whole-body lipid levels in the 2–3% (wet weight) range during the time of smoltification and migration to the estuary environment (Beckman *et al.*, 2000). Several other studies support the occurrence of low lipid concentrations in juvenile salmonids, especially those in the smolt stage (Table 1).

Redistribution of PCBs within an individual is also a potentially confounding factor. One recent study (Jørgensen *et al.*, 1999) found a 10-fold increase in PCBs in the liver of arctic char (a salmonid) that had been starved, even though the whole-body residue of total PCBs was unchanged. The lipid content of

Table 1. Whole-body lipid content in adult and smolt-stage salmonid species^a

Study	Species	Source	Lipid % wet wt.		
			Range (%)	<i>n</i>	Mean (%)
<i>Adults</i>					
1.	<i>Oncorhynchus mykiss</i>	Lab	8–12	3	8.4
2.	<i>Oncorhynchus mykiss</i>	Lab	6–10	36	8.3
3.	<i>Oncorhynchus tshawytscha</i>	Lab	7–11	4	9.2
4.	<i>Oncorhynchus mykiss</i>	Lab	8–10	16	9.2
5.	<i>Oncorhynchus mykiss</i>	Lab	8.4–8.5	3	8.5
6.	<i>Salvelinus fontinalis</i>	Lab	7.9–8.1	2	8.0
7.	<i>Salmo trutta</i>	Lab	6–16	8	10.8
8.	<i>Oncorhynchus mykiss</i>	Lab	7–13	51	9.7
<i>Smolt and pre-smolt fish</i>					
9.	<i>Oncorhynchus kisutch</i>	Wild/smolts	—	1	1.7
10.	<i>Oncorhynchus tshawytscha</i>	Hatch/smolts	2–3	16	2.4
11.	<i>Oncorhynchus tshawytscha</i>	Hatch/smolts	4–5	2 ^b	4.6
12.	<i>Oncorhynchus tshawytscha</i>	Hatch/smolts	3–5	5	3.8
13.	<i>Oncorhynchus tshawytscha</i>	Hatch/smolts	2–4	3 ^b	2.9
14.	<i>Oncorhynchus tshawytscha</i>	Wild/smolts ^c	1–2	6	1.1
15.	<i>Oncorhynchus tshawytscha</i>	Wild/smolts	1.6–2.5	5	2.1
16.	<i>Oncorhynchus tshawytscha</i>	Hatch/smolts	1.4–2.9	5	2.2
17.	<i>Oncorhynchus tshawytscha</i>	Wild/smolts	1.8–3.8	5	2.4
18.	<i>Oncorhynchus tshawytscha</i>	Wild/smolts	1–3	—	2
19.	<i>Oncorhynchus kisutch</i>	Wild/smolts	—	21 ^d	2.5
20.	<i>Oncorhynchus kisutch</i>	Hatch/smolts	—	30 ^e	3.8
21.	<i>Oncorhynchus kisutch</i>	Wild/presmolt	—	6 ^d	1.8
22.	<i>Oncorhynchus kisutch</i>	Hatch/presmolt	—	20 ^e	3.5

^a Fish considered adults if greater than 20 g wet wt. Lab indicates laboratory study and hatch are hatchery-reared fish. Mean (standard deviation) for studies 1–8 is 9.0% (0.9%). Citations 9–20 show lipid content for juvenile fish in the smolt stage. *n* = number of measurements; some samples were composites of several individuals. A dry weight to wet weight ratio of 0.2 was used to convert some values. 1. Beamish *et al.* (1986). 2. Hickie *et al.* (1989). 3. Shearer *et al.* (1997). 4. Reintz (1983). 5. Lieb *et al.* (1974). 6. Phillips *et al.* (1960). 7. Spigarelli *et al.* (1982). 8. Niimi and Oliver (1983). 9. Wood *et al.* (1960). 10–12. Collected at Soos Creek (Green River) Hatchery, WA. 1993, 1998, 2000. 13–16. Collected at Kellogg Is. (estuary) Green/Duwamish River, WA. 1993, 1998, 2000. 17. Collected from the Green River near Soos Creek Hatchery 2000. 18. Beckman *et al.* (2000). 19–22. Ludwig (1980). Numbers 10–17 are unpublished data from the Environmental Conservation Division, NMFS. *Oncorhynchus mykiss* previously *Salmo gairdneri*.

^b Were composed of 60 fish/sample.

^c Probably wild fish based on size (3–4 g).

^d Were composed of 10 fish/sample.

^e Were composed of 4–5 fish sample.

muscle decreased from 7.1% to 0.3%, presumably causing a mobilization of the PCBs to other lipid-containing organs, such as the liver, which exhibited only a modest change in lipid content. Kidney and brain PCBs also increased 2–3-fold in starved individuals. Toxicologically, this is an important observation for salmonids. These species are known to exhibit large declines in muscle lipid content during smoltification (Sheridan *et al.*, 1983), which would make juveniles in the estuary susceptible to large increases in PCBs in the liver and other organs. Additionally, because muscle tissue is the main lipid storage organ for salmonids, starvation will reduce muscle lipids as the fish use these energy stores, causing PCBs to be redistributed to other tissues. This would be expected during conditions of low food supply, which salmon may encounter during the winter in open water when food resources are more limited. It is expected that as total whole-body lipid declines, the lipid-normalized PCB concentration will increase, allowing for more of the PCBs to occur in the free state and increase the potential for toxicity at the site of action. As

discovered by many authors, reduction in lipid levels in salmonids does not appear to decrease the amount of whole-body PCBs (see Lieb *et al.*, 1974; Gruger *et al.*, 1975; Jørgensen *et al.*, 1999) but leads to a redistribution of these compounds to lipid-rich tissues.

The relationship between the PCBs found in whole body and that in liver appears to be highly variable and related to whole-body lipid content. Because variable lipid has such a large effect on the concentration of PCBs in various organs, tissue values reported here for juvenile salmonids are based on whole-body concentrations.

METHODS

Selection of studies

Several databases were examined to identify studies for consideration in this analysis. These include the US EPA database AQUIRE, Jarvinen and Ankley (1999) and Niimi (1996). The criteria for including studies in this analysis were:

1. The species examined was a member of the salmonidae family.
2. Results were from a controlled laboratory study.
3. The biological response in one or more treatments was statistically different from that in the control.
4. Tissue concentrations were reported or exposure was by injection or dietary uptake.
5. The life stage was relevant (fry to adults).
6. Individuals were exposed only to PCBs and only to a mixture (e.g. Aroclor 1254).

All studies that met the criteria were included. Studies that demonstrated biological effects for other life-cycle phases (e.g. eggs or embryos) were not included because they were not relevant for protecting juveniles in the estuary. The main focus was on sublethal responses. If mortality was included, an acute to chronic ratio of 10 was applied, which is standard for equating a lethal response to a sublethal response (McCarty and Mackay, 1993; Chapman *et al.*, 1998; Duke and Taggart, 2000).

Without additional data it cannot be determined if studies showing no significant effects had the statistical power to detect adverse effects or if the biological endpoint selected was not sensitive to the action of PCBs. In either case, these studies were deemed not useful for determination of adverse tissue concentrations in salmonids. This criterion is based, in part, on the relative costs of type I (false positives) versus type II (false negatives) errors inherent in hypothesis testing (Peterman, 1990). In assessing impacts to natural resources, particularly endangered species, type II errors are far more costly than type I errors and must be minimized.

Three studies that examined only one dose (concentration) of PCBs were included in Table 2. Two of these studies examined endpoints other than enzyme induction (Folmar *et al.*, 1982; Jørgensen *et al.*, 1999). The rest of the one-dose studies identified generally examined effects on enzyme systems in rainbow trout (Sivarajah *et al.*, 1978; Förlin, 1980; Voss *et al.*, 1982; Celander and Förlin, 1995; Celander *et al.*, 1996; Förlin *et al.*, 1996; Blom and Förlin, 1997). All of these studies exposed rainbow trout to one very high dose of PCBs (all at $100 \mu\text{g g}^{-1}$, except Förlin, 1980; $500 \mu\text{g g}^{-1}$). This group of studies all used injection (except Voss *et al.*, 1982; dietary) as a means to introduce PCBs. Because Melancon and Lech (1983) examined enzyme induction in rainbow trout at several concentrations and demonstrated a statistically significant response at $0.15 \mu\text{g g}^{-1}$ wet weight, the other one-dose studies were considered as a group. One study (Sivarajah *et al.*, 1978) was selected as representative of this group of one-dose studies because it demonstrated a statistically significant increase in the activity of several enzymes in addition to a significant decrease in steroid hormones.

Table 2. Tissue residue effect concentrations for salmonids^a

Study	Species	Time (days)	Route	Result/ endpoint	Lipid %		PCB tissue		
					wet wt.	dry wt.	conc for effect	µg g ⁻¹ lipid	
1. Melancon and Lech (1983)	Trout	b/5	Inject ^c	Inc. enzyme activity	9.0	45	0.15	0.75	1.7
2. Folmar et al. (1982)	Coho	b/60	Inject ^c	Alt. thyroid hormones	3.8	20	0.11	0.56	2.8
3. Bills et al. (1981)	Trout	30/30	Water ^c	Dec. LC50 to toxics	9.0	45	0.28	1.40	3.1
4. Berlin et al. (1981)	Lake trout	176/176	Diet and water ^c	Inc. mortality ^d	4.2	21	0.15	0.77	3.6
5. Bills and Marking (1977)	Trout	30/30	Water ^c	Dec. LC50 to toxics	9.0	45	0.46	2.3	5.1
6. Mayer et al. (1977)	Coho	260/260	Diet ^e	Inc. thyroid activity	9.0	45	0.59	3.0	6.6
7. Nestel and Budd (1975)	Trout	330/330	Diet ^e	Inc nephrosis & hepatocytes	11.4	57	1.3	6.5	11.4
8. Jørgensen et al. (1999)	Char	b/141	Diet ^e	Inc. fin erosion, alt. liver lipid	3.4	21	0.50	2.5	12.1
9. Chen et al. (1986)	Trout	180/180	Diet ^e	Dec. vitellogenin	9.0	45	1.5	7.5	16.7
10. Fisher et al. (1994)	Atlantic	2/176	Water ^f	Dec. growth	4.2	21	1.1	5.5	26.2
11. Thuvander et al. (1993)	Trout	b/63	Inject ^g	Abnormal spleen, liver, thymus and immune system	9.0	45	26.6	133	296 ^h
12. Mauck et al. (1978)	Brook trout	128/128	Water ^c	Inc. mortality ^d	4.2	21	12.5	63	298
13. Sivarajah et al. (1978)	Trout	b/28	Inject ^c	Inc. enzyme activity	9.0	45	75	375	833
14. Cleland et al. (1988)	Trout	360/360	Diet ^c	Dec. growth /dec. hormones	9.0	45	150	750	1667
15. Leatherland and Sonstegard (1978)	Coho	90/90	Diet ⁱ	Dec. growth, dec. thyroid hormones	9.0	45	250	1250	2778
				Geo mean			2.3	11.3	31.9
				10th Percentile			0.14	0.68	2.35
				25th Percentile			0.28	1.4	3.64
				Median			1.1	5.5	12.1

^aValues are for the lowest observed effect residue (LOER) in wet weight, dry weight, and lipid normalized concentrations. Whole-body lipid values in wet and dry weight. Estimated tissue and lipid values in bold (see text). Route is the method of administration of PCBs. Time in days is the length of time for exposure (First entry) and time allowed for responses to develop (second entry).

[tissue] wet weight $\times 5 =$ [tissue] dry weight. Trout = *Oncorhynchus mykiss*, coho = *Oncorhynchus kisutch*, char = *Salvelinus alpinus*, Atlantic = *Salmo salar*, brook trout = *Salvelinus fontinalis*; lake trout = *Salvelinus namaycush*. Percentiles determined with statistical program JMP[®] by SAS.

^bIndicates one administration of PCBs, except Folmar et al. (1978), two injections 10 days apart and Sivarajah et al. (1978), four injections over 4 weeks.

^cIndicates exposure to Aroclor 1254.

^dValue reflects adjustment with acute/chronic ratio (see text).

^eAroclor 1260.

^fEqual mix of Aroclors 1016, 1221, 1254, 1260.

^gClophen A50.

^hPCB tissue residues reported by author in µg g⁻¹ lipid.

ⁱ1:4 Mix of Aroclor 1242 and 1254.

Determination of tissue residues

Eight of the 15 studies reported whole-body tissue concentrations and were used without modification. Studies that contained information on the amount of PCBs injected or concentrations in the diet were used according to the following assumptions about accumulation. These assumptions regarding tissue residues from dietary uptake or injection are supported with the discussion presented below. In contrast, studies that exposed fish to water borne PCBs were not included unless tissue residues were reported. The determination of tissue residues from water exposure would introduce extreme uncertainty because the bioconcentration factor (BCF) for PCB congeners varies about 10000 fold and there is no one BCF for Aroclor mixtures (Bremle *et al.*, 1995). Moreover, accumulation from water can be highly variable due to such factors as variable water concentrations and the types of PCB congeners present in water. Uptake from water may also be more variable than the other routes when temperature, stress (behaviour), and dissolved organic carbon content are not constant or controlled. In contrast to this, many studies indicate fairly predictable tissue residues for both dietary uptake and injection.

Studies that reported tissue concentrations generally expressed them as wet weights. The predicted tissue concentrations based on injection or dietary exposure were also expressed as wet weights. A conversion factor of 5 was used for converting all wet weight concentrations to dry weight concentrations ([tissue] wet weight $\times 5 =$ [tissue] dry weight). Dry weights were then lipid normalized because lipid content is a major factor that controls the expression of toxicity, which was discussed above.

Injection studies

One study (Monosson *et al.*, 1994) reported that liver concentrations of a tetrachlorobiphenyl in white perch were about one-third to one-fifth of the injected concentration. These results are supported by Melancon *et al.* (1989) who also reported that the ratio of PCB liver concentrations to the injected concentration was about 0.3. Thuvander and Carlstein (1991) injected Clophen A50 into rainbow trout and reported whole-body PCB concentrations that ranged from 50% to 80% of the intended concentration. Similar results were reported by Guiney and Peterson (1980), who found that whole-body concentrations of 2, 5, 2', 5' tetrachlorobiphenyl in rainbow trout were about 75% of the injected dose. They also found a similar distribution of this PCB for various tissues (skin, viscera and carcass) when given orally and injected, indicating that injection studies are a reasonable way to introduce PCBs to salmonids. Based on these studies and the variability encountered, it was concluded that the whole-body tissue concentration for total PCBs would be best represented by a tissue residue that was 75% of the injected dose. Using the high end of this range (75%) will produce a higher tissue concentration associated with adverse effects compared to values derived from a lower value (e.g. 50%). We support the use of injection studies for PCB exposure because of the similarity in tissue distribution as that for dietary studies, the lack of metabolism, and the length of time needed for responses to develop.

Dietary studies

Many studies have demonstrated that salmonids absorb about 50% of the available PCBs in their diet. One recent study (Madenjian *et al.*, 1999) on coho salmon reported the efficiency of retention for various PCB congeners ranged from 38% to 56% for a dietary route of uptake. Similar results were also reported by Gruger *et al.* (1975, 1976) for coho salmon and by Opperhuizen and Schrap (1988) for guppies and other fish species (see references cited therein). In a long-term study with rainbow trout, Lieb *et al.* (1974) fed trout PCB-laden pellets for 32 weeks. Fish grew from 0.8 g to approximately 75 g and the percent retention of PCBs was determined to be 68%. The authors also determined that the ratio between the wet weight PCB concentration in fish and the PCB concentration in dry food was 0.54. Based on the studies listed above, the whole-body wet weight tissue concentration of PCBs in fish was assumed to be one-half of the

dietary dose. For example, fish in the study by Chen *et al.* (1986) were fed pellets containing $3 \mu\text{g g}^{-1}$ of PCBs; hence the resulting wet weight tissue concentration in fish was assumed to be $1.5 \mu\text{g g}^{-1}$. (Fish pellet concentrations are almost always dry weight values.)

RESULTS AND DISCUSSION

Normalization of tissue concentrations with lipid content

In general, adult salmon from lab studies have a higher whole-body lipid content (approximately 9.0% of wet weight) (Tables 1 and 2) than juvenile chinook from the field (approximately 1–2% of wet weight). Because of the high variability in lipid content found in salmonids at different life stages (Brett, 1995) and the toxicological implications, the tissue effect threshold is presented in terms of the lipid-normalized concentration.

Most of the studies did not report lipid content in their test fish. For those studies, a predicted whole-body lipid value was generated from an analysis of literature values. For adults, eight laboratory studies with salmonids were used (citations 1–8 in Table 1), which produced a mean (S.D.) lipid value of 9.0% (0.9%) of tissue wet weight. This value was then used to determine lipid-normalized tissue concentrations for the laboratory studies in Table 2. For fry and juveniles, data from Wood *et al.* (1960) and Higgs *et al.* (1995) were used, which generated a mean (S.D.) value of 4.2% (0.8%), $n = 16$. The whole-body lipid content for the fish used in Folmar *et al.* (1982) was estimated from data presented in Table 1 for cultured coho smolts. Lipid content for Jørgensen *et al.* (1999) was estimated using the data from Phillips *et al.* (1960) who reported whole-body lipid levels for another *Salvelinus* species that was starved for essentially the same length of time (144 versus 141 days).

Determination of the RET from literature values

Fifteen studies showing sublethal biological effects in salmonids exposed to PCBs passed all criteria and were included in Table 2. The lipid-normalized tissue concentrations (lowest observed effect residue, LOER) from Table 2 are plotted in Figure 1 as a function of the cumulative percent contribution by rank

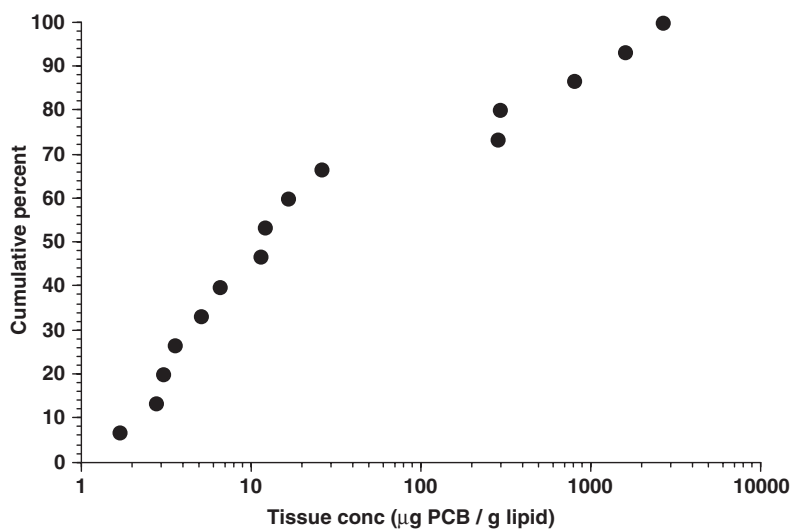


Figure 1. Cumulative distribution for tissue residue studies. Plot shows the cumulative distribution in rank order of all 15 studies (Table 2) used in the tissue residue analysis. Abscissa shows lowest observed effect tissue residue (LOER) for a given study.

order. This curve takes into account the variability produced by the different endpoints, statistical limitations of each study, and other factors such as variable time allowed for responses to develop and differences among species. The high variability in tissue concentrations associated with these LOERs is likely due to the various modes of action for PCBs. For example, enzyme induction and hormone alteration, would likely occur at tissue concentrations below that for growth impairment due to the different physiological processes that would be impaired.

All of the concentrations reported in Table 2 are 'effect' concentrations determined by analysis of variance (ANOVA), meaning that a significant biological response was observed at this tissue concentration. These values are termed LOERs meaning that these are the lowest tissue concentrations (residues) in the experiment where statistically significant effects were observed. The lipid-normalized tissue concentration considered protective against biological effects in juvenile salmonids migrating through the estuary was chosen as the 10th percentile of all the studies listed. This means that 90% of all studies were expected to exhibit a higher 'effect' concentration. A low percentile of all listed studies is an appropriate benchmark for protecting individual juvenile salmonids from sublethal effects that could decrease their long-term survival. This approach of selecting a low percentile in a series of ranked values is similar to that employed by the US EPA for determining national water quality criteria (Stephan *et al.*, 1985).

The results from Table 2 indicate that the 10th percentile value of all studies considered valid in the determination of a residue effect threshold for salmonids is $2.4 \mu\text{g PCB g}^{-1}$ lipid. Tissue residues below this are considered relatively protective for juvenile salmonids migrating through urban estuaries. This tissue concentration may indicate the potential for adverse effects in adult salmon as well. This threshold value is presented in Table 3, to show how different levels of lipid will affect the dry weight concentration. As noted in Figure 1, most of the studies reported effects in the range of 2 to $20 \mu\text{g g}^{-1}$ lipid. One study (Leatherland and Sonstegard, 1978) appears to be an outlier in relation to all other studies in Table 2 due to the high concentration reported for effects. The concentration reported by these authors ($250 \mu\text{g g}^{-1}$ wet wt.) is higher than the concentration generally associated with mortality and reduced growth in fish (Niimi, 1996). A recent exhaustive review of the literature concerning the responses of aquatic organisms to PCB exposure (Niimi, 1996) supports the assessment presented in Table 2, concluding that biochemical and cellular changes generally occur in fish when total PCB concentrations are in the high ppb to low ppm wet weight range.

Assuming that these 15 studies are a reasonable representation of most sublethal responses by salmonids to PCBs, it can be assumed that this curve (Figure 1) represents all such studies and any studies that would be conducted in the future. Considering that the tissue residues in Table 2 span the entire range from almost background to almost lethal, it is not surprising that additional studies should fall in this range. It is also likely that the next 10 or 15 studies will be distributed over this range, and will not be clumped at any

Table 3. RET for PCBs in salmonids^a

RET $\mu\text{g g}^{-1}$ lipid	Whole-fish lipid (% dry wt.)	Whole-fish lipid (% wet wt.)	RET ng g^{-1} wet wt.	RET ng g^{-1} dry wt.
2.4	5	1	24	120
2.4	10	2	48	240
2.4	15	3	72	360
2.4	20	4	96	480
2.4	25	5	120	600
2.4	30	6	144	720
2.4	35	7	168	840
2.4	40	8	192	960

^a Lipid-normalized RET for PCBs from Table 2. RET converted to whole body wet and dry weights based on lipid content.

particular concentration. This likely reflects the variability in experimentation and the different modes of action responsible for the observed effects.

Because the percentile values are based on rank order, the lowest values (e.g. 10th percentile) should not change dramatically with the addition of new results, unless they are relatively low. For example the 10th percentile concentration changed from 2.4 to 2.2 $\mu\text{g PCB g}^{-1}$ lipid with and without the concentration (1667 $\mu\text{g g}^{-1}$ lipid) reported by Cleland *et al.* (1988). In this case, the change in the 10th percentile is due to the addition of one more study (increasing the number of studies), not the value of the LOER (1667 $\mu\text{g g}^{-1}$ lipid).

As noted above, selecting a low percentile of all studies to determine a concentration for protection is an approach used by the US EPA (Stephan *et al.*, 1985). Analysis of the data presented in Table 2 using the EPA's algorithm for determining the final chronic value (FCV) produces a value of 1.7 $\mu\text{g g}^{-1}$ lipid. An alternative approach may have been to select a statistic, such as the geometric mean or median of the data, and apply a safety factor for converting the LOER data to a 'no effect' value (NOER). Considering the high variability in this dataset (Table 2), a safety factor of 10 would have been appropriate and is supported by other such applications (Chapman *et al.*, 1998; Duke and Taggart, 2000). Such an approach would have produced a similar threshold concentration (e.g. the geometric mean of 31.9 divided by 10 = 3.2 $\mu\text{g g}^{-1}$ lipid) (Table 2). (The same calculation with the median value for all studies in Table 2 equals 1.2 $\mu\text{g g}^{-1}$ lipid.) Therefore, it is not necessarily the first few studies in Table 2 that determine the RET; all of the studies in Table 2 contribute to the determination of the threshold value. The 10th percentile approach was selected because it is consistent with that used by other agencies; however, the 'safety-factor' approach is also well supported and in this case produces a similar value.

Uncertainty in the assumptions

Because the lipid content, wet to dry weight conversion factor, and the amounts of PCBs present in tissue from dietary and injection studies were estimated, a discussion of the uncertainty around each factor is warranted.

The mean lipid content for adults in Table 1 was used for nine of the 15 studies in Table 2. Several studies in Table 1 indicate that the lipid content for adults in laboratory studies varies between 6% and 16%. The coefficient of variation (CV) for the eight mean values listed in Table 1 is only 10%, indicating low variation among studies. This low CV indicates that the lipid content (9.0%) assumed for adult fish in any one of the Table 2 studies would likely be close to that value. The estimated lipid content used for the three studies with fry or pre-adults was more variable; however, the CV was <20%. The other two estimated values were based on fewer studies, but were likely close to actual values. The lipid content used for BSAF determinations was based on the data for wild, smolt-stage salmonids, which exhibited whole-body lipid levels in the 1–3% (wet weight) range (Table 1). Most studies of salmonids in the smolt stage demonstrate consistently low lipid values.

Another uncertainty concerns the use of total lipids when normalizing tissue concentrations. Lipids are composed of different classes (e.g. polar and non-polar) that may vary in proportion to the total amount present. Without information about the distribution of the various PCB congeners in the different lipid classes and the relative proportions of these lipid classes, there is some uncertainty regarding the use of a total lipid correction. However, even though PCB congeners may exhibit differential lipid-class partitioning (Ewald *et al.*, 1998), the toxicological significance of such partitioning is not known. Also, because we are concerned with one group of related species (salmonids), large differences in the partitioning of congeners and their relative effects as a result of such partitioning are not expected.

Whole-body tissue concentrations for PCBs were estimated in seven of the 15 studies. Three of these studies introduced PCBs by injection and four were by ingestion. The variability in the assumption made for dietary uptake is considered very low. Based on several studies cited above and comparable studies cited

in these publications, there is general agreement for a dietary uptake efficiency of approximately 50% for salmonids and several other fish species. The amount of variability associated with the injection mode of PCB administration is less certain due to the general lack of data. According to the studies cited above, the amount of PCBs retained by salmonids after injection ranges from 25% to 75%. Based on this variability and the influence that two of these studies had on the determination of the 10th percentile RET (Table 2), it was concluded that an assumption of 75% retention of the injected dose was reasonable. It is noteworthy that most of the studies where tissue residues were estimated occurred in the upper 50th percentile of all studies listed in Table 2.

A factor of five for converting wet weights to dry weights is standard, and low variability is usually encountered. This factor was used by Jarvinen and Ankley (1999) in their tissue residue database and it is also used by the EPA (Stephan *et al.*, 1985). Another source of uncertainty is the length of time for exposure. The longer an organism is exposed, the more likely it is to exhibit an adverse effect for a given tissue concentration. However, it is apparent in Table 2 that the long- and short-term studies are fairly evenly divided above and below the median tissue concentration.

The type of PCB mixture may also produce uncertainty in the analysis due to variable toxicity. Mayer *et al.* (1977) tested three fish species exposed to four different Aroclor mixtures and found a large range in LC50 values (10–100-fold) depending on the period of exposure and species. For the present study, 11 of the 15 studies examined Aroclor 1254, two studies used other mixtures (Clophen A50 and Aroclor 1260), and the other two exposed fish to combinations of different Aroclors (Table 2). Application of the RET and SET values generated here must consider the Aroclor profiles determined for tissue and sediment samples and their potential toxicity differences.

Ideally, a regression analysis producing an ER_p is preferred for determining adverse effects (e.g. ER_{10} ; ER stands for the 'effective residue', effective meaning sublethal; p represents the proportion responding). This is in contrast to the NOER/LOER concept (or NOEC/LOEC for exposure concentrations), which is determined by ANOVA. These values (LOER and NOER) are often information poor because they are dependent on the quantal nature of allocating exposure concentrations and sound experimental design with sufficient statistical power to avoid false negatives (i.e. accepting the null hypothesis of 'no treatment effect' when in fact an effect exists, but it cannot be detected with the current experimental design). If exposure concentrations are too far apart or few replicates are used in the experimental design, the LOER value determined by ANOVA may severely overestimate the true threshold value. In contrast, the ER_p value is determined directly with the dose–response curve and is a good statistical representation of the response, especially when a low proportion (e.g. ER_{10}) of the population is considered. None of the studies in Table 2 were sufficient to produce a regression equation linking exposure or tissue residues with a biological effect. Also, many of these studies examined only two or three concentrations that differed by up to an order of magnitude, leading to large gaps between the NOER and LOER values.

Case study: bioaccumulation of PCBs in juvenile salmon from the Duwamish River

Tissue residues

Determining the amount of PCBs accumulated by juvenile salmonids migrating through an urban estuary provides some unique challenges. The best approach would be to examine bioaccumulation in each river or estuary system of concern after determining concentrations in each compartment (water, sediment, prey and fish). Unfortunately, data for many systems are lacking and only a few are thoroughly studied. The following is one example of how to characterize bioaccumulation of PCBs in juvenile chinook salmon from an urban estuary.

In the Puget Sound area, most of the available data on PCB concentrations in migrating juvenile salmon make no distinction between wild and hatchery-reared fish. Recent data indicate that salmon raised in hatcheries have significant amounts of PCBs that likely come from the pellets they are fed (Gina Ylitalo,

Table 4. PCB concentrations in juvenile chinook salmon (*O. tshawytscha*) collected in the Green/Duwamish River^a

Data	Site	Year	Type	Mean PCB (ng g ⁻¹ dry)	n comp	Mean wet wt. (g)	n size
1.	Green River hatchery	1989	Whole body	687 (63)	4	5.2 (1.3)	122
2.	Kellogg Is.	1989	Whole body	960 (297)	5	5.5 (2.5)	215
3.	Green River hatchery	1993	Whole body	410 (14)	2	4.9 (1.4)	42
4.	Kellogg Is.	1993	Whole body	650 (252)	3	6.1 (1.2) ^b	42
5.	Green River hatchery	2000	Whole body	78 (14)	5 ^c	5.0	^d
6.	Fish trap — wild fish	2000	Whole body	42 (14)	14 ^c	4.0 (1.3)	26
7.	Kellogg Is. — wild fish	2000	Whole body	194 (137)	18 ^c	4.8 (1.1)	28
8.	Slip 4	2000	Whole body	1095 (1265)	8 ^c	4.8 (1.2)	15
9.	Kellogg Is.	1986/87	Stomach	3000 (350)	2	—	—
10.	Green River hatchery	1989/90	Stomach	550 (387)	2	—	—
11.	Kellogg Is.	1989/90	Stomach	1639 (638)	6	—	—
12.	Green River hatchery	1993	Stomach	600	1	—	—
13.	Kellogg Is.	1993	Stomach	2700 (1345)	3	—	—
14.	Green River hatchery	1986/87	Liver	290 (35)	1	—	—
15.	Kellogg Is.	1986/87	Liver	2600 (560)	3	—	—
16.	Green River hatchery	1989	Liver	215 (35)	2	—	—
17.	Kellogg Is.	1989	Liver	2167 (802)	3	—	—
18.	Green River hatchery	1993	Liver	243 (7)	3	—	—
19.	Kellogg Is.	1993	Liver	1077 (236)	3	—	—

^aSite of collection was the Duwamish estuary (Kellogg Island or Slip 4), Green River (Soos Creek) hatchery, or from a fish trap upstream of the hatchery. Mean values along with respective standard deviation are shown. All entries are for hatchery-reared fish, except for 6 and 7. *n* comp is the number of composite samples analysed for PCBs. Each whole-body composite contained 5–10 individual fish; liver composites contained \approx 60 livers (30 for McCain *et al.*, 1990), and composites for stomach contents were variable. *n* size is the number of fish weighed for the mean wet weight determination. Stomach contents for two sampling years (1989 and 1990) were pooled. The dry to wet weight ratio = 0.20 for whole body, 0.21 for liver and 0.17 for stomach contents. Data for 1989/90 from Varanasi *et al.* (1993) and values for 1993 and 2000 are unpublished data from the Environmental Conservation Division, NMFS. Data for 1986/87 from McCain *et al.* (1990).

^bFor the 1993 data, the mean wet weight for fish collected in the lower river was significantly larger ($p < 0.001$) than the mean for hatchery fish.

^cAre mix of individual fish and composites of 5–10 fish.

^dValue determined by hatchery.

NMFS, pers. comm.). Other sources of PCBs, such as maternal transfer, may also contribute to the overall tissue burden; however, this has rarely been examined. The most extensive dataset available for this exercise in the Puget Sound area is for the Green/Duwamish River system in Washington State (Table 4). Over the past several years, the NMFS has sampled juvenile salmon at Kellogg Island in the Duwamish River (Figure 2) because it is in the estuary, downstream of most of the industrial area, provides suitable salmon habitat, and is accessible for beach seining. Most of the samples from Kellogg Island contain a mixture of wild and hatchery-reared fish; however, most of the juvenile chinook outmigrating in the river system come from the hatcheries (approximately 75% of the 11 million chinook salmon that migrate down this river) (Varanasi *et al.*, 1993). Only recently (spring 2000) have all hatchery fall-run chinook in the Green/Duwamish River been marked, allowing wild fish to be distinguished from hatchery fish.

The first step in this analysis was to determine how much of the total PCBs were accumulated at the hatchery and how much were accumulated in the river (Table 5). The point of this exercise was to provide an estimate of the PCB concentrations that would occur in wild chinook, which is the main focus for ESA protection in this river. For the samples taken in 1989 and 1993, it was determined that on average, juvenile chinook captured at Kellogg Island accumulated approximately 1800 ng of PCBs for each 5–6 g fish in the river after leaving the hatchery. The tissue concentrations for fish from the two independent sampling periods were remarkably similar (310 and 320 ng g⁻¹ dry wt.), leading to an average concentration of 315 ng g⁻¹ dry wt.

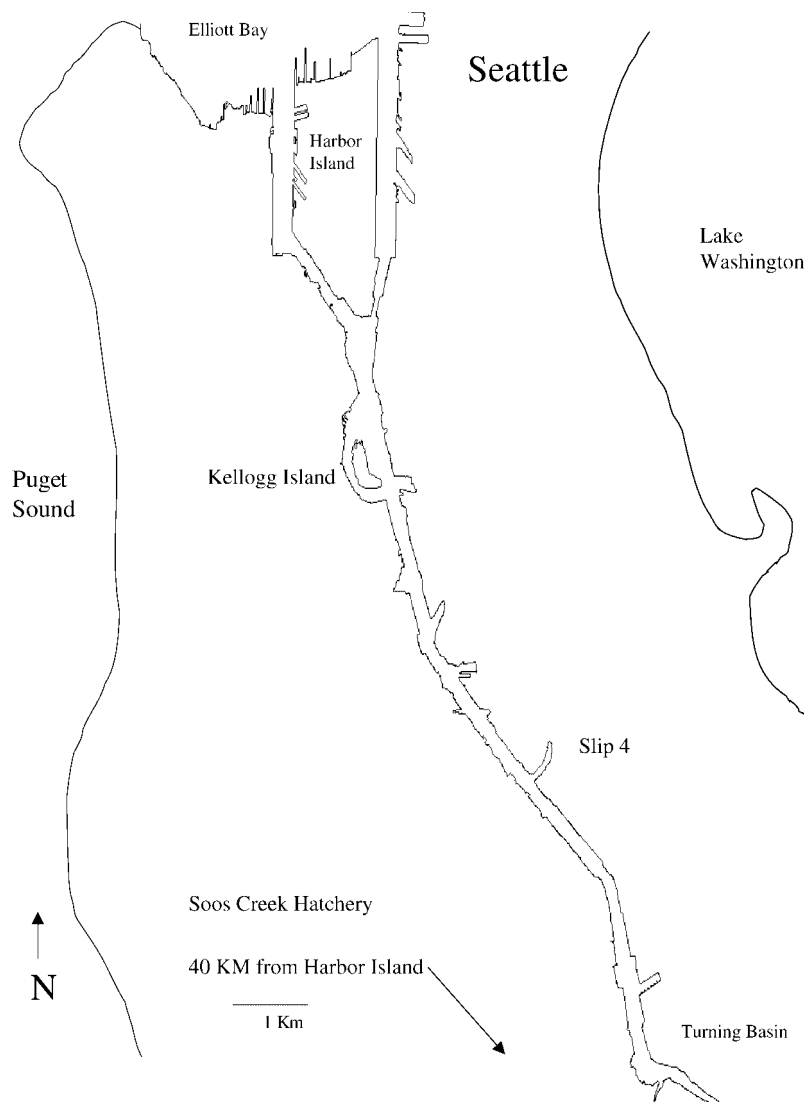


Figure 2. Map showing the Duwamish Waterway. Harbor Island is just south and west of downtown Seattle.

A recent study that examined only wild juvenile chinook in the Duwamish River confirms these results, although the average tissue concentration was lower (Table 5). Wild fish collected a few hundred metres upstream of the Soos Creek Hatchery exhibited low PCB concentrations (42 ng g^{-1} dry wt.), whereas wild fish collected at Kellogg Island contained total PCBs ranging from 100 to 475 ng g^{-1} dry wt. (NMFS 2000, unpublished data) (Table 4, Figure 3). (The Soos Creek hatchery is approximately 35 km upstream of the Duwamish estuary.) It is also important to note that the whole-body tissue concentrations for PCBs in hatchery fish declined between 1989 and 2000 (Table 4). This trend may be due to declining PCB levels found in the fish that are processed into fish pellets; however, additional samples are needed to assess the variability of these concentrations.

Table 5. Accumulation of PCBs in juvenile chinook in the Duwamish River estuary^a

Pair	Source	Year	Mean weight of fish (g)	Total PCBs (ng g ⁻¹)	PCBs total (ng)	Conc. from estuary exposure (ng g ⁻¹)
<i>Average fish</i>						
1a	Hatchery	1989	5.2	687	3572	
1b	Kellogg Is.	1989	5.5	960	5280	310
2a	Hatchery	1993	4.9	410	2010	
2b	Kellogg Is.	1993	6.1	650	3965	320
3a	Hatchery	2000	5.0	78	390	
3b	Slip 4	2000	4.8	1095	5256	1014
4a	Wild — Upstream	2000	4.0	42	168	
4b	Wild — Kellogg Is.	2000	4.8	194	931	159
<i>Maximum value</i>						
1c	Kellogg Is.	1989	5.5	1300	7150	651
2c	Kellogg Is.	1993	6.1	940	5734	611
3c	Slip 4 ^b	2000	3.1	4021	12465	3895
4c	Wild — Kellogg Is. ^b	2000	3.5	475	1663	427

^a Hatchery is Soos Creek (Green River) hatchery, Kellogg Island and Slip 4 are sites in the Duwamish estuary. Wild denotes naturally reared fish collected near the hatchery and in the estuary. All pairs are hatchery fish, except number 4. Concentration of PCBs in fish from estuary determined by subtracting total ng for hatchery fish from total ng in estuary fish and dividing by weight of estuary fish. The same calculation was done for the maximum value. All concentrations as dry weight. Source for the 1989 data is Varanasi *et al.* (1993) and the 1993 and 2000 data are from an unpublished report from NMFS, Environmental Conservation Division.

^b Denotes individual fish, all others values are means from composites of several fish.

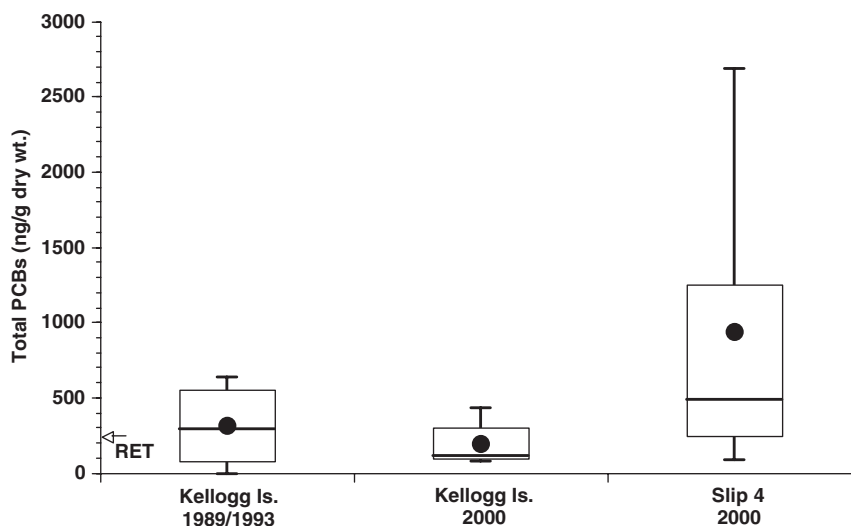


Figure 3. Box plot for total whole-body PCBs accumulated in the Duwamish River estuary by juvenile chinook salmon. All values were determined by subtracting out hatchery contribution. Circle (●) is the mean for the data. Lines forming the top and bottom of box represent 75th and 25th percentiles of the data. Line in the middle of box is 50th percentile or median. Whiskers above and below the box are the 90th and 10th percentiles. The arrow at 240 ng g⁻¹ marks the tissue effect threshold (RET) concentration for a 10% lipid content (dry wt.). Fish from Kellogg Island 2000 were all wild, whereas those from 1989/93 were a mixture of wild and hatchery fish. Slip 4 samples consisted entirely of hatchery fish (see Tables 4 and 5 for more details).

Additional data collected in May 2000 to support the current analysis examined juvenile chinook sampled at a site of high PCB contamination in the Duwamish River system. This site, called Slip 4, is approximately 4 km upstream of Kellogg Island. Whole-body concentrations as high as 4000 ng g^{-1} dry wt. were observed in fish from this site, with the mean value approximately 3 times that for Kellogg Island fish (Figure 3, Table 5) (NMFS 2000, unpublished data). Due to the high whole-body PCB concentrations, 75% of all fish sampled from Slip 4 were higher than the RET. Based on the approximate lipid content for these fish (10% dry wt. Table 1), the average PCB concentration was $10.1 \mu\text{g PCBs g}^{-1}$ lipid with the maximum value of $39.0 \mu\text{g PCBs g}^{-1}$ lipid. These concentrations fall in the middle of the values for the 15 studies listed in Table 2.

A box plot of these data are presented in Figure 3 for the 1989 and 1993 data ($n = 8$ composite samples) and the May 2000 data for wild fish from Kellogg Island and hatchery fish from Slip 4. The high variability in this plot is likely due to the differences in the amount of time the fish spent in the lower river (Duwamish waterway) after leaving the hatchery, fish size, and the inability to differentiate between wild and hatchery fish (first box).

The data in Table 5 and Figure 3 demonstrate that juvenile chinook salmon were accumulating PCBs in the lower Duwamish River, which is supported by the concentrations observed in stomach contents (Table 4). The total PCBs in the stomachs of the fish in the lower river were 3–4 times higher than those found in hatchery fish. Also noteworthy are the liver concentrations for PCBs. The concentrations of total PCBs in the livers of juvenile chinook salmon collected in the lower river were 4–10 times higher than those concentrations noted for hatchery fish (Table 4), yet the difference in whole-body concentrations were not as large. This apparent increase in liver PCBs is consistent with the study of Jørgensen *et al.* (1999) with arctic char showing a redistribution of PCBs to the liver when the whole-body lipid content declined in fish that were starved. As noted in Table 1, the smolted fish collected from the lower river generally have much lower lipid levels than fish from the hatchery.

Sediment concentrations

A study of 326 sediment samples from 88 strata (non-overlapping areas of the sediment surface) in the Duwamish estuary was used to determine the mean of all PCB sediment concentrations (Industrial Economics, 1998; Jennie Bolton, pers. comm.). A minimum variance unbiased (MVU) estimator (Gilbert, 1987) for lognormal values was used to determine the mean, which was found to be 324 ng g^{-1} dry wt. ($n = 326$), with the 95% confidence interval (CI) of $261\text{--}439 \text{ ng g}^{-1}$. The variance for this mean was 5500. These mean values were calculated using all values from the study, ignoring the strata that were sampled. Applying the same MVU estimator to the means of all strata ($n = 88$) produced a similar mean value (319 ng g^{-1} dry wt.), but a much higher variance (7365). In most strata only a few samples were taken, often producing high within-strata variability. The median of these data, which describes the 50th percentile, was determined to be 74.1 ng g^{-1} dry wt. using an MVU estimator (Gilbert, 1987). Because this is a lognormal distribution, the median is expected to be less than the mean. The MVU estimator was also used to determine the mean sediment value based on organic carbon content. The mean of all TOC-normalized sediment concentrations ($n = 326$) was found to be 19665 ng g^{-1} OC. The 95% CI for this mean was $16\,580\text{--}25\,798 \text{ ng g}^{-1}$ OC. The mean (S.D.) for TOC at these 326 stations in the Duwamish waterway was 1.5% (0.75%). The PCB chemistry data and their distribution as determined with the Normal Density Function (Gilbert, 1987) are presented in Figure 4.

Biota-sediment accumulation factor (BSAF)

The next step was to determine the average BSAF value for juvenile chinook salmon in this estuary system. The mean tissue concentration of PCBs acquired in the estuary at Kellogg Island (315 ng g^{-1}) divided by the approximate average dry weight lipid content of 10% (Table 1) for in-river smolts, gives a value of 3150 ng

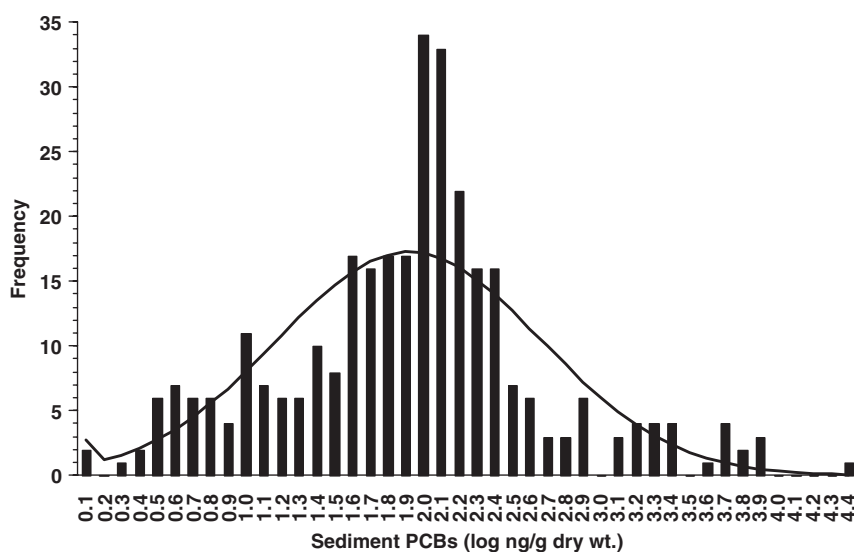


Figure 4. Distribution plot of PCB sediment concentrations in the Duwamish River. Each bar represents the number of samples falling in a $\log 0.1 \text{ ng g}^{-1}$ interval. The solid line is the expected frequency based on the normal density function. Data from Industrial Economics (1998) and Peggy Krahn, Environmental Conservation Division, NMFS.

PCBs g^{-1} lipid. The lipid-normalized tissue concentration (3150 ng g^{-1} lipid) divided by the organic-carbon-normalized sediment concentration ($19\,665 \text{ ng g}^{-1}$ OC) yields a mean (S.D.) BSAF of 0.16 (0.13) for PCBs accumulated by fish collected in 1989 and 1993 in the estuary. This is a value based on averages and it assumes that juvenile chinook forage in an 'average' manner over the river, which may not be a valid assumption. The mean (S.D.) BSAF for wild chinook captured during May 2000 at Kellogg Island was 0.10 (0.07).

Using the average TOC-normalized sediment concentrations for Slip 4 ($n = 8$), the average BSAF for hatchery fish collected from Slip 4 in May of 2000 was 0.03, with a maximum value of 0.13. Considering the high variability in both sediment ($302\,000 \text{ ng g}^{-1}$ OC) and tissue ($10\,140 \text{ ng g}^{-1}$ lipid) concentrations (CV for sediment = 200% and tissue = 100%) and the indeterminate time for residence, the BSAF was expected to be highly variable in this localized inlet of the river. If one very contaminated sediment concentration is eliminated from the analysis for Slip 4, the mean sed_{oc} becomes $90\,400 \text{ ng g}^{-1}$ OC and the CV reduces from 200% to 63%. Based on this sed_{oc} , the mean BSAF for juvenile chinook from Slip 4 would be 0.11, with a maximum value of 0.44. Based on an ANOVA, this BSAF for Slip 4 fish (= 0.11) was not statistically different than that for hatchery-reared fish (BSAF = 0.16) collected in, 1989/93 or wild fish (BSAF = 0.10) collected at Kellogg Island in May 2000.

Because the Soos Creek hatchery released a large number of fish just 5 days before NMFS sampled the lower Duwamish River in 2000, low BSAF values were expected. Consequently, the BSAF values reported for hatchery fish are likely for a short exposure period (e.g. 5 days). It is not known how long the wild fish were in the estuary. A review by Thorpe (1994), indicates that juvenile chinook salmon spend an average of 30 days, and up to 45 days, in the estuary before moving out to more open water. Based on this average residence time, higher BSAFs than reported here are expected for juvenile chinook in the Duwamish estuary.

The BSAF for chinook was in contrast to that for shiner perch (*Cymatogaster aggregata*) collected concurrently with the salmon at Slip 4. These fish contained up to $10 \mu\text{g g}^{-1}$ dry wt. of total PCBs and their

BSAF averaged 0.35, which is 3 times higher than the chinook values (NMFS, 2000, unpublished data). Interestingly, the catch per unit effort (CPUE) for juvenile chinook in Slip 4 was about 5–10 times higher than that for Kellogg Island on the same day, indicating extensive habitat use of this area by juvenile chinook. Due to the large number of juvenile chinook captured in the Slip 4 area of the Duwamish, it is not clear which sampling site is more representative of PCB exposure in this estuary.

Determination of the SET

Once the RET was established, it became important to relate this value to a sediment concentration. Because sediment in urban areas can be a major source of PCBs to biota, the areas with high sediment concentrations need to be identified so appropriate action can be taken to control their contribution to the overall burden found in migrating salmon and the food webs on which they depend.

Using the tissue residue data (Table 2), predictions were generated for sediment concentrations below which adverse biological effects in migrating juvenile salmon would be minimal. This was done by solving for the sediment concentration using the BSAF formula (equation (2)). The PCB sediment concentrations that are not expected to cause appreciable adverse effects in the 'average' juvenile chinook migrating through the Duwamish estuary are listed in Table 6. Several values are listed as a function of total organic carbon in the sediment. Assuming an average sediment TOC of 1.5% and a BSAF of 0.16, the SET would be 225 ng g^{-1} dry wt. which is approximately 90 ng g^{-1} lower than the average sediment concentration for the Duwamish River and 10–30 times higher than sediment concentrations found in non-urban areas around Puget Sound and the West Coast of the United States (Malins *et al.*, 1982; McCain *et al.*, 1988; Stehr *et al.*, 1997). The BSAF value ($= 0.16$) determined for the 1989/93 samples was selected because it was generated with the most data. It should be noted that this BSAF ($= 0.16$) was not statistically different from the BSAFs generated for hatchery fish from Slip 4 or wild fish collected at Kellogg Island in May 2000. Additional studies at different locations in the estuary with fish in residence for variable lengths of time are needed to confirm or refine this value.

The Endangered Species Act explicitly protects most individuals, not just the 'average' individual. When assessing the PCB tissue concentrations found in migrating juvenile salmon, an upper percentile (e.g. 90th or 95th percentile) of the amount accumulated in the estuary is appropriate to evaluate biological effects, not the average concentration. For the fish collected at Kellogg Island, the 95th percentile PCB tissue concentration was 650 ng g^{-1} dry wt., which is 3 times higher than the RET (Figure 3). For Slip 4, the 95th percentile concentration ($= 3062 \text{ ng g}^{-1}$ dry wt.) was 13 times higher than the RET, with most fish above the threshold value. The same consideration should be used when assessing the SET. The 95th percentile

Table 6. SET concentration for total PCBs based on two BSAF values^a

Tissue threshold (RET) $\mu\text{g g}^{-1}$ lipid	Sediment TOC % dry wt.	SET ng g^{-1} dry wt. (BSAF = 0.16)	SET ng g^{-1} dry wt. (BSAF = 0.32)
2.4	1.0	150	75
2.4	1.5	225	113
2.4	2.0	300	150
2.4	2.5	375	188
2.4	3.0	450	225
2.4	3.5	525	263
2.4	4.0	600	300

^aLipid-normalized RET for PCBs from Table 2. SET determined with equation (2). Sediment PCB concentrations determined as ng g^{-1} OC but presented as ng g^{-1} dry wt. Values correspond to an organic-carbon-normalized sediment concentration (sed_{OC}) of $15.0 \mu\text{g g}^{-1}$ OC for the mean BSAF ($= 0.16$) and $7.5 \mu\text{g g}^{-1}$ OC for the 95th percentile BSAF ($= 0.32$) (see text).

BSAF value for fish (wild and hatchery mixed) collected at Kellogg Island was 0.32 (1989 and 1993 data; Table 5), which was essentially the same value for Slip 4 fish (=0.34). The 95th percentile BSAF for wild juvenile chinook collected in May 2000 was 0.24. If the 95th percentile BSAF (= 0.32) is used instead of the mean value (= 0.16), the SET for the Duwamish system would be 113 ng g^{-1} dry wt. (TOC = 1.5%) (Table 6).

Based on the distribution in Figure 4, the sediment concentrations in the Duwamish River comprising the upper 10th percentile are from 2 to 25 times higher than the mean concentration. The high and variable tissue concentrations seen in the results from Kellogg Island and Slip 4 (Figure 3), suggest that some of the fish were not feeding in an 'average' fashion in their migration down the river. Obviously, some fish were feeding more often in areas with high sediment concentrations of PCBs compared to areas with low concentrations indicating differential habitat utilization. This was highly evident based on the fish from Slip 4, whose whole-body tissue residues were up to 10 times higher than the Kellogg Island fish. The distribution of whole-body PCB tissue concentrations for fish sampled in the Duwamish estuary indicates that only a small percentage of the fish that visited Slip 4 would likely visit or be collected at Kellogg Island. Consequently, the marked differences in body burdens found in juvenile salmon from different sites (e.g. Slip 4 versus Kellogg Island) suggests that these fish are exhibiting some degree of site fidelity during their residence in the estuary.

Even though the overall mean sediment concentration for the Duwamish system was relatively low, there were sites with very high concentrations (e.g. Slip 4), which obviously contributed to the elevated tissue residues seen in some samples. Reducing the areal extent of these hotspots will likely reduce the amount of PCBs accumulated. The main goal should be to achieve an acceptable mean or median sediment concentration with a relatively low variance that would increase the probability that juvenile chinook migrating through this system would exhibit tissue residues below the RET. One way to accomplish this would be through an iterative process of reducing high sediment concentrations in parts of the river and measuring the resulting concentrations in fish tissues, which may be a reasonable approach for a river system such as the Duwamish. For example, lowering the highest 10% of the sediment concentrations to 50 ng g^{-1} in the Duwamish River (Figure 4) reduces the overall mean sediment concentration by 31% and the variance by 53%.

One limitation for this framework of establishing an effect threshold is that cumulative effects are not considered. The only way to accurately determine the relationship between biological effects and a particular class of contaminants is with controlled laboratory studies. Because the results in Table 2 are from laboratory studies that examined only PCBs, there is no assessment of the interactive effects that are expected from other toxicants found in environmental matrices. Consequently, biological effects in juvenile salmon may occur at even lower PCB tissue concentrations than reported here. Because of this, the proposed SET may actually be lower when the additive or synergistic effects of additional toxicants, such as PAHs, DDT, toxic metals, and organometallics, are considered. For example, the studies by Arkoosh *et al.* (1998) and Varanasi *et al.* (1993) are field studies in the Duwamish River system that demonstrate adverse biological effects in juvenile salmon at PCB tissue concentrations in the $0.5\text{--}1 \mu\text{g g}^{-1}$ dry wt. range ($2.5\text{--}10 \mu\text{g g}^{-1}$ lipid; for 10–20% dry wt. lipid, see Table 1), which are generally lower than comparable values for these laboratory-generated endpoints presented in Table 2. These two studies suggest that the observed biological responses (survival, growth, disease resistance) in field-exposed fish may be lower for a given PCB concentration due to the effects of additional toxicants. Chemical analysis of the fish from the Duwamish (Table 4; 1989/93 data) also detected PAHs in their stomach contents ranging from 10 to $169 \mu\text{g g}^{-1}$ wet wt. (Varanasi *et al.*, 1993), indicating very high exposure to these important contaminants. It is toxicologically valid to suggest that the results of these field studies may be due to the additive or synergistic relationship among all bioaccumulated contaminants; however, we lack the data necessary to assess such interactions. This is a feature that should be incorporated into future studies and ecological risk assessments.

SUMMARY

The residue effect threshold (RET) for salmonids exposed to PCBs was determined to be $2.4 \mu\text{g PCB g}^{-1}$ lipid. This was generated by calculating the 10th percentile of 15 research studies that examined biological responses in various species of salmonids exposed to PCBs. Tissue concentrations below this value (RET) are expected to protect juvenile salmon migrating through urban estuaries from adverse effects due to PCB exposure. In some cases it may be desirable to convert the RET to an equivalent sediment effect threshold (SET) for use in regulating exposure. Analysis of lipid-normalized tissue residues and comparison with the RET is the preferred method for assessing adverse effects of PCBs on juvenile salmon; however, if bioaccumulation can be characterized in an estuary of interest, then the BSAF approach and generation of a SET may be a useful way to protect against injury.

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REFERENCES

- Arkoosh MR, Casillas E, Huffman P, Clemons E, Evered J, Stein JE, Varanasi U. 1998. Increased susceptibility of juvenile chinook salmon (*Oncorhynchus tshawytscha*) from a contaminated estuary to the pathogen *Vibrio anguillarum*. *Transactions of the American Fisheries Society* **127**: 360–374.
- Beamish FWH, Hilton JW, Niimi E, Slinger SJ. 1986. Dietary carbohydrate and growth, body composition and heat increment in rainbow trout (*Salmo gairdneri*). *Fish Physiology Biochemistry* **1**: 85–91.
- Beckman BR, Larsen DA, Sharpe C, Lee-Pawlak B, Schreck CB, Dickhoff WW. 2000. Physiological status of naturally reared juvenile spring chinook salmon in the Yakima river: seasonal dynamics and changes associated with smolting. *Transactions of the American Fisheries Society* **129**: 727–753.
- Berlin WH, Hesselberg RJ, Mac MJ. 1981. Chlorinated hydrocarbons as a factor in the reproduction and survival of lake trout (*Salvelinus namaycush*) in Lake Michigan. US Fish and Wildlife Service Technical Papers Number 105, 11–22.
- Bills TD, Marking LL. 1977. Effects of residues of the polychlorinated biphenyl Aroclor 1254 on the sensitivity of rainbow trout to selected environmental contaminants. *Progressive Fish Culturist* **39**: 150.
- Bills TD, Marking LL, Mauck WL. 1981. Polychlorinated biphenyl (Aroclor 1254) residues in rainbow trout: effects on sensitivity to nine fishery chemicals. *North American Journal of Fisheries Management* **1**: 200–203.
- Blom S, Förlin L. 1997. Effects of PCB on xenobiotic biotransformation enzyme activities in the liver and 21-hydroxylation in the head kidney of juvenile rainbow trout. *Aquatic Toxicology* **39**: 215–230.
- Bremle G, Okla L, Larsson P. 1995. Uptake of PCBs in fish in a contaminated river system: bioconcentration factors measured in the field. *Environmental Science and Technology* **29**: 2010–2015.
- Brett JR. 1995. Energetics. In *Physiological Ecology of Pacific Salmon*, Groot C, Margolis L, Clarke WC (eds). UBC Press: Vancouver; 3–68.
- Celander M, Förlin L. 1995. Decreased responsiveness of the hepatic cytochrome P450 1A1 system in rainbow trout (*Oncorhynchus mykiss*) after prolonged exposure to PCB. *Aquatic Toxicology* **33**: 141–153.
- Celander M, Stegeman JJ, Förlin L. 1996. CYP1A1-, CYP2B-, and CYP3A-like proteins in rainbow trout (*Oncorhynchus mykiss*) liver: CYP1A1-specific down-regulation after prolonged exposure to PCB. *Marine Environmental Research* **42**: 283–286.
- Chapman PM, Fairbrother A, Brown D. 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. *Environmental Toxicology and Chemistry* **17**: 99–108.
- Chen TT, Reid PC, Van Beneden R, Sonstegard RA. 1986. Effect of Aroclor 1254 and Mirex on estradiol-induced vitellogenin production in juvenile rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* **43**: 169–173.
- Cleland GB, McElroy PJ, Sonstegard RA. 1988. The effect of dietary exposure to Aroclor 1254 and/or Mirex on humoral immune expression of rainbow trout (*Salmo gairdneri*). *Aquatic Toxicology* **2**: 141–146.

- Di Giulio RT, Benson WH, Sanders BM, Van Veld PA. 1995. Biochemical mechanisms: metabolism, adaptation, and toxicity. In *Fundamentals of Aquatic Toxicology*, 2nd edn, Rand GM (ed.). Taylor & Francis Pub: Washington, DC; 523–561.
- Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA, Paquin PR. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environmental Toxicology and Chemistry* **10**: 1541–1583.
- Duke LD, Taggart M. 2000. Uncertainty factors in screening ecological risk assessments. *Environmental Toxicology and Chemistry* **19**: 1668–1680.
- Ewald G, Bremle G, Karlsson A. 1998. Differences between Bligh and Dyer and Soxhlet extractions of PCBs and lipids from fat and lean fish muscle: implications for data evaluation. *Marine Pollution Bulletin* **36**: 222–230.
- Fairchild WL, Swansburg EO, Arsenault JT, Brown SB. 1999. Does an association between pesticide use and subsequent declines in catch of Atlantic salmon (*Salmo salar*) represent a case of endocrine disruption? *Environmental Health Perspectives* **107**: 349–357.
- Fisher JP, Spitsbergen JM, Bush B, Jahan-Parwar B. 1994. Effect of embryonic exposure on hatching success, survival, growth, and developmental behavior in landlocked Atlantic salmon, *Salmo salar*. In *Environmental Toxicology and Risk Assessment: 2nd volume, ASTM STP 1216*, Gorsuch JW, Dwyer FJ, Ingersoll CG, La Point TW (eds). American Society for Testing and Materials: Philadelphia; 298–314.
- Folmar LC, Dickhoff WW, Zaugg WS, Hodgins HO. 1982. The effects of Aroclor 1254 and no. 2 fuel oil on smoltification and seawater adaptation of coho salmon (*Oncorhynchus kisutch*). *Aquatic Toxicology* **2**: 291–299.
- Forbes VE, Calow P. 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? *Environmental Toxicology and Chemistry* **18**: 1544–1556.
- Förlin, L. 1980. Effects of Clophen A50, 3-methylcholanthrene, pregnenolone-16 α -carbonitrile and phenobarbital on the hepatic microsomal cytochrome P-450 dependent monooxygenase system in rainbow trout, *Salmo gairdneri*, of different age and sex. *Toxicology and Applied Pharmacology* **54**: 420–430.
- Förlin L, Blom S, Celander M, Sturve J. 1996. Effects on UDP glucuronosyl transferase, glutathione transferase, DT-diaphorase and glutathione reductase activities in rainbow trout liver after long-term exposure to PCB. *Marine Environmental Research* **42**: 213–216.
- Gilbert RO. 1987. *Statistical Methods for Environmental Pollution Monitoring*. Van Nostrand Reinhold Company: New York.
- Giesy JP, Kannan K. 1998. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): implications for risk assessment. *Critical Reviews in Toxicology* **28**: 511–569.
- Gruger EH, Hruba T, Karrick NL. 1976. Sublethal effects of structurally related tetrachloro-, pentachloro-, and hexachlorobiphenyl on juvenile coho salmon. *Environmental Science and Technology* **10**: 1033–1037.
- Gruger EH, Karrick NL, Davidson AI, Hruba T. 1975. Accumulation of 3,4,3',4' tetrachlorobiphenyl, 2,4,5,2',4',5' and 2,4,6,2',4',6' hexachlorobiphenyl in juvenile coho salmon. *Environmental Science and Technology* **9**: 121–127.
- Guiney PD, Peterson RE. 1980. Distribution and elimination of a polychlorinated biphenyl after acute dietary exposure in yellow perch and rainbow trout. *Archives of Environmental Contamination and Toxicology* **9**: 667–674.
- Hickie BE, Dixon DG, Leatherland JF. 1989. The influence of the dietary carbohydrate:lipid ratio on the chronic toxicity of sodium pentachlorophenate to rainbow trout (*Salmo gairdneri* Richardson). *Fish Physiology and Biochemistry* **6**: 175–185.
- Higgs DA, MacDonald JS, Levings CD, Dosanjh BS. 1995. Nutrition and feeding habits in relation to life history stage. In *Physiological Ecology of Pacific Salmon*, Groot C, Margolis L, Clarke WC (eds). UBC Press: Vancouver; 161–315.
- Industrial Economics. 1998. Duwamish Waterway Sediment Characterization Study Report. Prepared for the Damage Assessment Center, NOAA.
- Jarvinen AW, Ankley GT. 1999. *Linkage of Effects to Tissue Residues: Development of a Comprehensive Database for Aquatic Organisms Exposed to Inorganic and Organic Chemicals*. SETAC Press: Pensacola.
- Jones PD, Kannan K, Newsted JL, Tillitt DE, Williams LL, Giesy JP. 2001. Accumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin by rainbow trout (*Oncorhynchus mykiss*) at environmentally relevant dietary concentrations. *Environmental Toxicology and Chemistry* **20**: 344–350.
- Jørgensen EH, Bye BE, Jobling M. 1999. Influence of nutritional status on biomarker response to PCB in the Arctic char (*Salvelinus alpinus*). *Aquatic Toxicology* **44**: 233–244.
- Lassiter RR, Hallam TG. 1990. Survival of the fittest: implications for acute effects of lipophilic chemicals on aquatic populations. *Environmental Toxicology and Chemistry* **9**: 585–595.
- Leatherland JF, Sonstegard RA. 1978. Lowering of serum thyroxine and triiodothyronine levels in yearling coho salmon, *Oncorhynchus kisutch*, by dietary Mirex and PCBs. *Journal Fisheries Research Board Canada* **35**: 1285–1259.
- Lieb AJ, Bills DD, Sinnhuber RO. 1974. Accumulation of dietary polychlorinated biphenyls (Aroclor 1254) by rainbow trout (*Salmo gairdneri*). *Journal Agriculture and Food Chemistry* **22**: 638–642.

- Ludwig BW. 1980. A morphological and biochemical comparison of artificially and naturally reared salmonids. Masters thesis, University of British Columbia.
- Madenjian CP, Schmidt LJ, Chernyak SM, Elliott RF, Desorcie TJ, Quintal RT, Begnoche LJ, Hesselberg RJ. 1999. Variation in net trophic transfer efficiencies among 21 PCB congeners. *Environmental Science and Technology* **33**: 3768–3773.
- Malins DC, McCain BB, Brown DW, Sparks AK, Hodgins HO, Chan S-L. 1982. Chemical contaminants and abnormalities in fish and invertebrates from Puget Sound. NOAA Technical Memorandum OMPA 19.
- Mauck WL, Mehrle PM, Mayer F. 1978. Effects of the polychlorinated biphenyl Aroclor 1254 on growth, survival, and bone development in brook trout (*Salvelinus fontinalis*). *Journal Fisheries Research Board Canada* **35**: 1084–1088.
- Mayer FL, Mehrle PM, Sanders HO. 1977. Residue dynamics and biological effects of polychlorinated biphenyls in aquatic organisms. *Archives of Environmental Contamination and Toxicology* **5**: 501–511.
- McCain BB, Brown DW, Krahn MM, Myers MS, Clark RC Jr, Chan S-L, Malins DC. 1988. Marine pollution problems, North American West Coast. *Aquatic Toxicology* **11**: 143–162.
- McCain BB, Malins DC, Krahn MM, Brown DW, Gronlund WD, Moore LK, Chan S-L. 1990. Uptake of aromatic and chlorinated hydrocarbons by juvenile chinook salmon (*Oncorhynchus tshawytscha*) in an urban estuary. *Archives of Environmental Contamination and Toxicology* **19**: 10–16.
- McCarty LS, Mackay D. 1993. Enhancing ecotoxicological modeling and assessment. *Environmental Science and Technology* **27**: 1719–1728.
- Melancon MJ, Lech JJ. 1983. Dose–effect relationship for induction of hepatic monooxygenase activity in rainbow trout and carp by Aroclor 1254. *Aquatic Toxicology* **4**: 51–61.
- Melancon MJ, Turnquist KA, Lech JJ. 1989. Relation of hepatic microsomal monooxygenase activity to tissue PCBs in rainbow trout (*Salmo gairdneri*) injected with ¹⁴C PCBs. *Environmental Toxicology and Chemistry* **8**: 777–782.
- Monosson E, Fleming WJ, Sullivan CV. 1994. Effects of the planar PCB 3,3',4,4'-tetrachlorobiphenyl (TCB) on ovarian development, plasma levels of sex steroid hormones and vitellogenin, and progeny survival in the white perch (*Morone americana*). *Aquatic Toxicology* **29**: 1–19.
- Nestel H, Budd J. 1975. Chronic oral exposure of rainbow trout (*Salmo gairdneri*) to a polychlorinated biphenyl (Aroclor 1254): pathological effects. *Canadian Journal of Comparative Medicine* **39**: 208–215.
- Niimi AJ. 1996. PCBs in aquatic organisms. In *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*, Beyer WH, Heinz GH, Redmon-Norwood AW (eds). Lewis Pubs: Boca Raton; 117–152.
- Niimi AJ, Oliver BG. 1983. Biological half-lives of polychlorinated biphenyl (PCB) congeners in whole fish and muscle of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* **40**: 1388–1394.
- NOAA. 1999. Endangered and threatened wildlife and plants; definition of 'harm'. National Oceanic and Atmospheric Administration. Federal Register, Vol. 64, No. 215, November 8, 1999, 60727–60731. 50 CFR Part 222.
- Opperhuizen A, Schrap SM. 1988. Uptake efficiencies of two polychlorobiphenyls in fish after dietary exposure to five different concentrations. *Chemosphere* **17**: 253–262.
- Peterman RM. 1990. Statistical power analysis can improve fisheries research and management. *Canadian Journal of Fisheries and Aquatic Sciences* **47**: 2–15.
- Phillips Jr AM, Livingston DL, Dumas RF. 1960. Effect of starvation and feeding on the chemical composition of brook trout. *Progressive Fish Culturist* **22**: 147–154.
- Reinitz G. 1983. Relative effect of age, diet, and feeding rate on the body composition of young rainbow trout (*Salmo gairdneri*). *Aquaculture* **35**: 19–27.
- Shearer KD, Silverstein JT, Dickhoff WW. 1997. Control of growth and adiposity of juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* **157**: 311–323.
- Sheridan MA, Allen WV, Kerstetter TH. 1983. Seasonal variation in the lipid composition of the steelhead trout, *Salmo gairdneri* Richardson, associated with the parr-smolt transformation. *Journal of Fish Biology* **23**: 125–134.
- Sivarajah K, Franklin CS, Williams WP. 1978. The effects of polychlorinated biphenyls on plasma steroid levels and hepatic microsomal enzymes in fish. *Journal of Fish Biology* **13**: 401–409.
- Spigarelli SA, Thommes MM, Prepejchal W. 1982. Feeding, growth, and fat deposition by brown trout in constant and fluctuating temperatures. *Transactions of the American Fisheries Society* **111**: 199–209.
- Stehr CM, Myers MS, Burrows DG, Krahn MM, Meador JP, McCain BB, Varanasi U. 1997. Chemical contamination and associated liver diseases in two species of fish from San Francisco Bay and Bodega Bay. *Ecotoxicology* **6**: 35–65.
- Stephan CE, Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA. 1985. *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses*. US EPA. Office of Research and Development, PB85-227049.
- Thorpe JE. 1994. Salmonid fishes and the estuarine environment. *Estuaries* **17**: 76–93.
- Thuvander A, Carlstein M. 1991. Sublethal exposure of rainbow trout (*Oncorhynchus mykiss*) to polychlorinated biphenyls: effect on the humoral immune response to *Vibrio anguillarum*. *Fish and Shellfish Immunology* **1**: 77–86.

- Thuvander A, Wiss E, Norrgren L. 1993. Sublethal exposure of rainbow trout (*Oncorhynchus mykiss*) to Clophen A50: effects on cellular immunity. *Fish and Shellfish Immunology* **3**: 107–117.
- US EPA. *AQUire Toxicity Information Retrieval (AQUIRE) Database*. US EPA, NHEERL, MED-Duluth, MN.
- van Wezel AP, de Vries DAM, Kostense S, Sijm DTHM, Opperhuizen A. 1995. Intraspecies variation in lethal body burdens of narcotic compounds. *Aquatic Toxicology* **33**: 325–342.
- Varanasi U, Casillas E, Arkoosh MR, Hom T, Misitano DA, Brown DW, Chan S-L, Collier TK, McCain BB, Stein JE. 1993. Contaminant exposure and associated biological effects in juvenile chinook salmon (*Oncorhynchus tshawytscha*) from urban and nonurban estuaries of Puget Sound. NOAA Technical Memorandum NMFS-NWFSC-8.
- Voss SD, Shelton DW, Hendricks JD. 1982. Effects of dietary aroclor 1254 and cyclopropene fatty acids on hepatic enzymes in rainbow trout. *Archives of Environmental Contamination and Toxicology* **11**: 87–91.
- Walker MK, Peterson RE. 1991. Potencies of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and biphenyl congeners, relative to 2,3,7,8 tetrachlorodibenzo-*p*-dioxin, for producing early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* **21**: 219–238.
- Wedemeyer GA, Saunders RL, Clarke WG. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. *Marine Fisheries Review* **42**: 1–14.
- Wood EM, Yasutake WT, Halver JE, Woodall AN. 1960. Chemical and histological studies of wild and hatchery salmon in fresh water. *Transactions of the American Fisheries Society* **89**: 301–307.