REGIONAL SEDIMENT EVALUATION TEAM PROCESS CHANGE FORM FOR THE SEDIMENT EVALUATION FRAMEWORK FOR THE PACIFIC NORTHWEST (SEF 2018)
DRAFT RSET ISSUE PAPER NO. 2025-01 – Revision of PCB Target Tissue Level for Juvenile Salmonids
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ISSUE/CHANGE: Revise PCB target tissue level for use in bioaccumulation testing for salmonids to address concerns with background concentrations and use of some sublethal endpoints in the original calculation (SEF 2018, Appendix E).

DISCUSSION:

Background: Target Tissue Levels (TTLs) are criteria concentrations in tissue against which bioaccumulation testing results can be compared to determine whether sediments are suitable for in-water disposal or pose a bioaccumulation risk and therefore require other disposal options. *The Sediment Evaluation Framework for the Pacific Northwest* (U.S. Army Corps of Engineers et al. 2018, hereafter SEF 2018) describes TTLs for protection of aquatic life (Table 8-5) and aquatic-dependent wildlife (Table 8-6) and includes a TTL of 1.4 µg/g lipid derived specifically to protect salmonids from threats posed by bioaccumulation of polychlorinated biphenyl (PCB) congeners.

Due to environmental weathering, differential uptake by biota, and uncertainties with analytical methods, total PCBs quantified using the total of 209 congeners is preferred over total PCB using Aroclor analysis (Oregon Department of Environmental Quality 2022). Failure to consider weathering in environmental investigations may significantly over or underestimate total PCB concentrations. To both accurately quantify and evaluate the risk of total PCBs as a chemical class, the total of 209 congeners are compared to the total PCB Aroclor thresholds.

The original juvenile salmonid TTL (U.S. Army Corps of Engineers et al. 2009) was derived from data presented in Meador et al. (2002) that included endpoints such as altered enzyme activity that may not result in adverse effects on growth, survival, or reproduction (typical endpoints in risk assessments) and the TTL may be at or near background concentrations for PCBs. This paper describes a re-evaluation of the juvenile salmonid TTL using endpoints more directly associated with adverse effects on fitness (focusing on growth and survival for the juvenile stage), including sublethal effects on sensitive transitional stages of development (effects that ultimately reduce growth and fitness).

Juvenile salmonids are exposed to PCBs from urban aquatic ecosystems (LWG 2006a, Johnson et al. 2013, LWG 2013, Lundin et al. 2021) while completing their physiological transition from freshwater to marine environments. Subyearlings are typically more vulnerable to effects of PCBs because of their greater residence time in estuaries compared to yearlings. During this exposure period, the fish are not only vulnerable to effects on growth and survival, but also to sublethal responses such as altered thyroid hormones, and disease susceptibility that can reduce their fitness. Alteration of any associated physiological functions, especially thyroid hormones, may substantially reduce the chances of successful smoltification and the ability to survive and grow in saltwater. Lipids are also a very important factor in regulating toxicological effects in juvenile salmonids. PCBs stored in lipids and mobilized during important transition stages have been shown to alter thyroid hormones and smoltification, which is described in more detail in Attachment 1. In this paper, PCB concentrations in fish tissue are normalized to lipid weight from wet weight or dry weight to better compare effect levels across species with different lipid levels.

Discussion: Data used for calculation of a salmonid TTL in the SEF was based on an evaluation from Meador et al. (2002). Meador et al. (2002) reviewed and used the lowest observed tissue residue (LOER) from 15 laboratory studies evaluating total PCB effects on salmonids (including trout species). These data were ranked using a cumulative distribution function to derive a residue effect threshold (10th percentile value of all studies) for salmonids of 2.4 µg/g lipid (or hazardous concentration percentile – HC10). To derive a more protective TTL for juvenile salmonids, the Regional Sediment Evaluation Team Bioaccumulation Subcommittee in the 2009 SEF (U.S. Army Corps of Engineers et al. 2009) used results from the 15 studies in a Species Sensitivity Distribution (SSD) to estimate a 5th percentile hazard concentration (HC05) of the SSD interpolated from a fitted distribution using the Burrlioz software program to fit the toxicity data using a Burr type III distribution (SEF 2018), an approach reviewed by Fox et al. (2021). The HC05 from this approach was 1.4 µg/g lipid (Sections 8.5.6 and E.1.4 of the 2018 SEF).

The Regional Sediment Evaluation Team re-evaluated the 2009 HC05 derivation because sublethal endpoints were included that have not been demonstrated to have adverse effects on survival, growth, or reproduction, and that may be at or near background values for juvenile salmonids. This is of note because the SEF states that in cases where the TTL is below background or detection limits, the default will be to compare to background concentrations or detection limits if background levels are not known. To better address this issue and address bioaccumulation risk to salmonids, we

1) identified potential background concentrations for juvenile chinook salmon in the lower Columbia and Willamette Rivers,
2) reviewed adverse effect levels reported in recently conducted laboratory and field studies on juvenile chinook salmon,
3) revised the SSD-derived TTL and calculated a HC05 TTL for salmonids using only those studies associated with adverse effects on fitness (including some sublethal effects), and
4) compared the revised HC05 data to a recent evaluation of PCB tissue concentrations associated with effects on a broad range of fish including non-salmonid species.

Summary of Re-Evaluation (see supporting information for details)

1) Regionally relevant background or reference for summed PCB concentrations:

- Subyearling chinook sampled at four sites outside the influence of known PCB sources in the lower Columbia and Willamette Rivers averaged about 0.7 (ranging from 0.3 to 1.0) microgram per gram (µg/g) lipid.
- Subyearling chinook sampled in the lower Willamette River in 2005 at a reference area from river mile 17 to 18 averaged 0.94 (ranging from 0.72 to 1.20) µg/g lipid.

- Subyearling chinook sampled in the lower Willamette River at an upriver reference site at river mile 16 averaged 1.3 (ranging from 1.1 to 1.6) µg/g lipid.
- Whole body smallmouth bass sampled in the lower Willamette River at river mile 15.9 averaged 1.1 µg/g lipid.

2) Regionally relevant summed PCB concentrations associated with effects:

- Subyearling chinook sampled in the lower Willamette River where known sources of PCBs, dichlorodiphenyltrichloroethane (DDT) and related compounds (collectively referred to as DDx), and polynuclear aromatic hydrocarbons (PAHs) occur experienced reduced growth (based on otolith measurements) at 3.6 µg/g lipid compared to fish sampled at upriver sites.
- Subyearling chinook dosed with a subset of PCBs, DDx, and PAHs in the laboratory experienced growth effects and increased disease susceptibility at 1.0 µg/g lipid.

3) HC05 derived using PCB tissue data on salmonids associated with adverse effects only:

- SSDs using PCB effects data on salmonids (focusing only on decrease in growth, altered thyroid hormones, and abnormalities) and estimated lipid values from eight of the 15 studies reviewed by Meador et al. (2002) resulted in a HC05 of 1.5 µg/g lipid. The lowest effect level used in the Meador et al. (2002) evaluation was from a study (Folmar et al. 1982) on yearling coho salmon where thyroid hormones were altered at PCB concentrations of 2.8 µg/g lipid. This study was retained in the re-evaluation and remains the lowest study with adverse effects.

4) Comparison to recent evaluation of PCBs in whole body fish tissue (including non-salmonid species) associated with adverse effects:

- Berninger and Tillitt (2019) modeled results from 31 laboratory and 24 field studies (22 fish species not specific to salmonids) and found fish lipid content was not used to independently normalize adverse effects in each study. However, the lowest concentration associated with adverse effects (mortality, growth, reproduction) in aggregate data was 3.0 µg/g lipid (140 µg/kg wet weight at an assumed average of 4.6% lipid).
- The same authors identified a lower limit of applicability in their model of 2.2 µg/g lipid (100 µg/kg wet weight at 4.6% lipid), below which adverse effects to mortality, growth or reproduction were not supported by the data used in their evaluation.
- Though some early life stage studies were used in this review, none included endpoints in juvenile salmonids associated with sensitive transitional stages of development (such as altering thyroid hormone levels during smoltification, which can affect growth).

PROPOSED LANGUAGE/ISSUE RESOLUTION: Revision of 2009 SSD TTL for PCBs in Table 8-5 and E-1, and Section E.1.3 to 1.5 µg/g lipid and adding a brief description of basis for the revised SSD TTL, language to be determined. This SSD TTL is for use in freshwater and estuary systems where juvenile salmonids could be present at any stage of their life cycle.

RECOMMENDATION:
This evaluation indicates a whole-body tissue TTL of 1.5 µg/g lipid for PCBs is above background and would be protective of bioaccumulation risk for juvenile salmonids. This value is recommended for adoption into the SEF for use in waters where juvenile salmonids could be present during any life stage. The value can be reviewed and revised periodically when new PCB tissue data become available using the SSD process described herein. We also recommend additional laboratory studies be conducted to evaluate the effects of PCBs on subyearling salmon at environmentally relevant lipid concentrations such as those levels expected during outmigration.

For the current revised TTL approach, it is expected that tissue data used in bioaccumulation testing will be analyzed using a high-resolution congener-specific method and total PCBs will be summed as total congeners. Lipid will be determined as described by the Bligh and Dyer (1959) chloroform/methanol extraction method, or the less toxic solvent system (Hara and Radin1978) using hexane/isopropanol in combination with gravimetric measurement of lipid.

REFERENCES:
See supporting information in Attachment 1

RESPONSE TO COMMENTS:

LIST OF COMMENTS/COMMENTERS:

FINAL DECISION:

Attachment 1. Supporting Information

Background/reference concentrations in whole body subyearling chinook or other whole-body fish outside of influence of known PCB sources:

- Whole body subyearling chinook were sampled by Johnson et al. (2013) at 14 locations in the lower Columbia River. Average concentrations from four sites above the confluence with the lower Willamette River were estimated at 0.7 (ranging from about 0.3 to 1.0) µg/g lipid, or in wet weight at 8.9 µg/kg (ranging from 3.3 to 12.5 µg/kg) with lipid levels measured in juvenile fish averaging 1.4% for the four sites (wet weight results approximated from graphed data presented for the Warrendale, Peirce Island, Franz Lake, and Mirror Lake sites). Total PCBs in tissues were represented as the sum of 45 congeners. These 45 PCB congeners are a subset of the total 209 congeners and represent those that are most common in the water column, are persistent and have the greatest potential for bioaccumulation.
- Whole body subyearling chinook were sampled from 10 locations in the lower Willamette River by Lundin et al. (2021). Total PCBs in samples from a reference site (G-Alt) at river mile 16 averaged 1.3 (ranging from 1.1 to 1.6) µg/g lipid, or 15.1 (ranging from 11.6 to 21.1 µg/kg) µg/kg wet weight at 1.3% lipid measured in wild-caught fish. Total PCBs in tissues were represented as the sum of 45 congeners. Note: there is a known source of PCBs that is being characterized by Oregon

Department of Environmental Quality at river mile 16.7 which could influence PCBs in fish from the G-Alt location.

- Whole body subyearling chinook were sampled by the Lower Willamette Group (LWG 2006a) as a part of the Portland Harbor Superfund Remedial Investigation in the lower Willamette River at four locations, including one reference location (T04) at river miles 17 to 18. Total PCBs in samples from this reference site (average of three replicates) was 0.94 µg/g lipid (17 µg/kg wet weight at 1.8% lipid). Total PCBs represented the sum of 209 congeners or co-elute values. Total Aroclor results were also measured in this study and were very similar to total congener results.
- Whole body smallmouth bass collected (LWG 2013) at various locations in the lower Willamette River including a reference area around river mile 15.9 and above. Total PCBs (represented by 209 congeners) were 1.1 µg/g lipid (50 µg/kg wet weight based on average whole fish lipid of 4.6%); 1% is more representative of sub-yearling salmonids.

Effects to subyearling Chinook in field and laboratory studies:

- Lundin et al. (2021) sampled subyearling chinook salmon from 10 sites in the lower Willamette River. PCBs, DDx, and PAHs were measured in whole body and stomach contents from these sites. Subyearling chinook are considered to feed and reside in the lower Willamette River (approximately river mile 0 to 12) for weeks or longer before transitioning to the lower Columbia River estuary, inhabiting the area for longer than other juvenile stages and therefore being more susceptible to exposure to PCBs and other contaminants. Otolith analysis was used to assess growth in subyearlings of natural origin at sampled sites. PCBs were elevated in whole-body samples collected near areas with elevated PCBs in sediment. Reduced growth was associated with higher tissue concentrations of some contaminants including higher-chlorinated PCBs. Average total PCBs (sum of 45 congeners) at the site with the highest whole-body fish concentrations was 8.6 (range 3.2 to 24) µg/g lipid, although reduced growth was observed at PCB concentrations as low as 3.7 µg/g lipid compared to reference fish (Lundin et al. 2021, 2024).
- Lundin et al. (2024) dosed subyearling chinook salmon through the diet in the laboratory (5 weeks to evaluate growth and an additional 23 days to evaluate disease susceptibility) with a mixture of 10 PCB congeners, 3 DDx compounds, and 13 PAHs (Lundin et al. 2021). These compounds were associated with growth effects on juvenile Chinook in the previous field study (Lundin et al. 2021), and dosing levels and analytes were selected based on stomach content analysis from juvenile Chinook salmon sampled in the Portland Harbor and Duwamish River Superfund sites (Lundin et al. 2024).The lipid content of these fish averaged 7.1% due to a high lipid laboratory diet. High lipid content could have mitigated some observed effects, yet growth (as measured by body mass and fork length) was significantly reduced in individuals in some treatment groups. Evidence of slower growth was also noted as measured by otolith analysis. Growth effects and disease susceptibility were reported in the most environmentally relevant dosing level which resulted in whole body PCB concentrations at 1.0 µg/g lipid (sum of 45 congeners), or 71 µg/kg wet weight at 7.1% lipid. Subyearling wild salmonids or their prey items tend to contain around 1 to 2 percent lipid, so when translated to 1% lipid, effects observed in the study

occurred around 10 µg/kg wet weight for PCBs (with co-occurrence of DDx and PAHs).

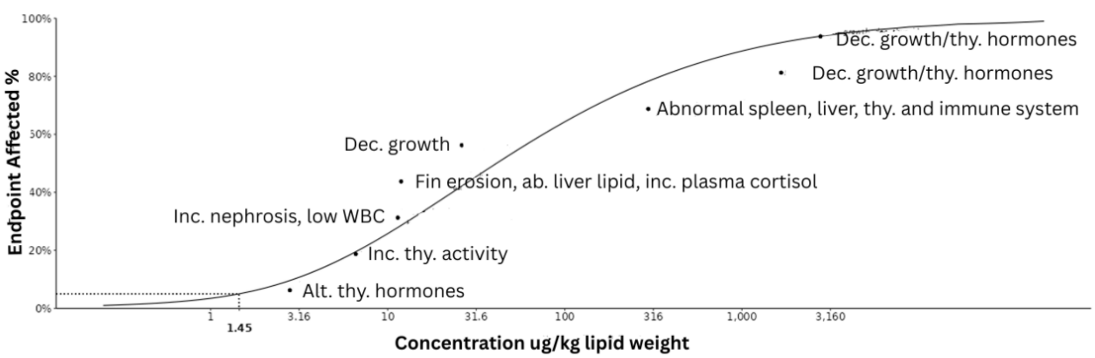
- For comparison to tissue residues in other studies, LWG (2006a) collected whole body subyearling chinook from areas within the lower Willamette River and found the average of total PCBs (sum of 209 congeners) was 9.7, 6.0, and 11 µg/g lipid from three sites within the Superfund designated portion of the Portland Harbor and 0.94 µg/g lipid for the upstream reference site (three replicates per site).

HC05 Derivation using PCB tissue data on salmonids

- Meador et al. (2002) reviewed studies evaluating the effects of PCBs on salmonids and derived data rules resulting in the selection of 15 studies for their analysis. The authors adjusted the 15 studies to make effect concentrations comparable. These adjustments included 1) estimating lipid concentrations (because most studies did not measure lipids directly); 2) estimating whole body PCB residues from injection- and diet-exposed dose studies when residues were not reported; and 3) estimating chronic effects using an acute to chronic uncertainty factor when mortality was the endpoint.
- Eight of the 15 studies reviewed by Meador et al. (2002) were selected for use in the re-analysis presented here. Effects in seven of the original 15 studies were not clearly linked to an adverse effect on survival or growth, or used an acute to chronic uncertainty factor, and thus were excluded in the re-analysis unless the effect was associated with sensitive development periods for early life stage fish. Endpoints incorporated from the eight accepted studies included alteration in thyroid hormones and activity (which effects juvenile salmon during critical stages of growth including preparation for transition to saltwater), reduced growth, fin erosion and other abnormalities to internal organs, and nephrosis. All studies primarily reported effect concentrations in wet weight and did not report lipid weight, so lipid values estimated for each study by Meador et al. (2002) were used in the re-evaluation. Values associated with effects from the eight studies ranged from 2.8 to 2,778 µg/g lipid. All studies dosed fish through diet or injection using PCBs as Aroclors.
- Although there is uncertainty with estimating lipid concentrations (see EPA 2003 for review of different types of lipids in tissues and inconsistencies in lipid analysis), our review considered lipid adjustment to be appropriate given high lipid diet and oil carrier solutions used in laboratory studies on juvenile salmonids that could mitigate adverse effects and are not reflective of diet or lipid in wild subyearling fish (see *Importance of lipid values in relation to PCB toxicity for juvenile salmonids* section below for a more detailed description of the mitigating effects of lipids).
- The process used by Meador et al. (2002) for estimating tissue residues for dietary and injection studies was retained in the re-analysis. Whole body concentrations were estimated as 75% of the dose for injection studies (1 study) and as 50% of the dose for dietary studies (3 studies). Supporting information for estimating tissue residues based on dietary and injection studies is described in Meador et al. (2002) and Berninger and Tillitt (2019).
- The HC05, or the 5th percentile of adverse effect data for salmonid survival, reproduction and growth, was calculated using the online application shinyssdtools version 0.1.1 (Dalgarno 2018, 2021, available at https://bcgov-env.shinyapps.io/ssdtools/) to fit and plot SSDs using a graphical user interface. This

same version is available in R as part of ssdtools version 1.0.2 (Thorley and Schwarz 2018). Fox et al. (2021) provides a recent review of SSD approaches including use of the tools.

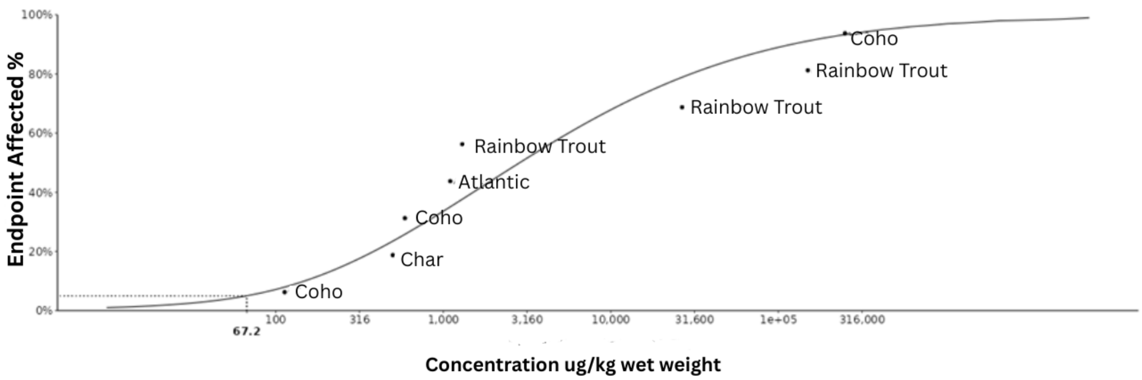
- For the re-analysis, we employed a model-averaging approach using maximum likelihood estimation to calculate a HC05 using the eight selected studies with sensitive effect endpoints for salmon. This method is considered an alternative to selecting a single best fit distribution, and, as reviewed by Fox et al. (2021) "…can reduce the uncertainty associated with fitting distributions to small data sets as well as providing some immunity to perturbations in HCx values due to the influence of a single sensitive data point."
- Using lipid-normalized effect concentrations estimated from the eight studies by Meador et al. (2002), we calculated a HC05 from the SSD by selecting the distributions that demonstrated acceptable fit (delta <2.0) using the Akaike Information Criterion (AIC) corrected for small sample sizes. Distributions that met the criteria included the log Gumbel, log logistic, and log normal distributions. These three distributions had the best fit when weighted by log Gumbel at 0.522, log normal at 0.285, and log logistic at 0.194 (weighted sum = 1.0). The figure below shows the average fit of the three distributions based on these weights.



- The lipid-normalized predicted HC05 based on the weighted distribution was 1.5 µg/g lipid.
- Benthic invertebrates such as clams and worms commonly used in bioaccumulation testing (and representative of the trophic level included in the diet of juvenile salmonids) vary in lipid concentrations yet typically contain about 1% to 2% lipid. To aid in the interpretation of bioaccumulation test results, the chart below translates the lipid-normalized PCB TTL of 1.5 µg/g lipid to a TTL in µg/kg wet weight using the measured % lipid of the test organism.

TTL (HC05) µg/g lipid	Test organism lipid (%)	TTL µg/kg wet wt
1.5	0.5	8
1.5	0.75	11
1.5	1.0	15
1.5	1.25	19
1.5	1.75	26
1.5	2.0	30

- To evaluate the variability of using lipid-normalized tissue effect levels in the calculation of the HC05, we also determined a HC05 from an SSD using the measured or estimated wet weight effect concentrations from the eight selected studies. Repeating the model-averaging approach on the wet weight data resulted in best fit for the log Gumbel, log logistic, and log normal distributions. The three distributions were weighted by log Gumbel at 0.487, log normal at 0.305, and log logistic at 0.208 (weighted sum = 1.0) and the average fit is depicted below.



- The predicted HC05 based on the weighted distributions was 67 µg/kg wet weight.
- The HC05 value of 67 µg/kg wet weight is above background and within the range of values where effects could occur (see effects section above). Translation of this wet weight-based HC05 to a lipid-normalized HC05 depends on the percent lipid value selected to represent the laboratory test fish in the eight selected studies. This is illustrated in the chart below, where a range of potential lipid content is used to convert to a lipid weight.

TTL (HC05) µg/kg wet weight	Organism lipid	TTL µg/g lipid weight
67	3.0%	2.2
67	4.2%	1.6
67	5.0%	1.3
67	6.0%	1.1
67	7.0%	1.0

- Meador et al. (2002) estimated a lipid value of 4.2% (SD = 0.8%) to represent juvenile salmon based on data from 16 laboratory tests. Using this lipid content, the HC05 wet weight value translates to a lipid weight TTL of 1.6 µg/g (see chart above) which is very similar to our proposed lipid-normalized TTL of 1.5 µg/g.
- More recent testing reveals a higher lipid content than 4.2% in juvenile salmonids used in controlled studies (Powell et al. 2002, Lundin et al. 2024). The higher lipid content may be more typical of fish used in laboratory testing due to the high fat content of dietary feed. This suggests the resulting 1.6 µg/g lipid TTL might be less protective for salmonids because higher lipid can act as a buffer to the effects of PCBs (as described in more detail below).

- Based on this re-analysis and resulting convergence of calculated HC05 values using both approaches as detailed above, we recommend using the study specific lipid-normalized approach described in Meador et al. (2002) in the SSD to calculate the HC05. By using the Meader et al. 2002 data, we calculated an HC05 of 1.5 µg/g lipid which maintains lipid as an important explanatory variable for observed effects throughout the analysis.

Data Review by Berninger and Tillitt (2019)

- Explored relationships between PCB exposure (as total Aroclors) and effects including growth in 22 fish species across 31 laboratory studies (adverse effects based on 30 datapoints for mortality, 17 for growth, 8 for reproduction) and 24 field studies.
- Both salmonids and non-salmonids were evaluated.
- Sensitivity distributions identifying probability of adverse effects were presented as wet weight regression thresholds. Data rules and adjustments to input data were similar to those used by Meador et al. (2002). Studies reporting lipid-normalized concentrations were converted to wet weight assuming an average 4.6% lipid content or by using study- or species-specific lipid concentrations when available.
- Data review provided a low effects value for PCBs (based on mortality, growth, or reproduction) for fish based on whole body residues at 140 µg/kg wet weight, or 3.0 µg/g lipid using a 4.6% average lipid content for fish as assumed in their study. For juvenile salmonids at 1% lipid content (a value more typical of wild subyearlings and their prey), this translates to 30 µg/kg wet weight.
- Review also provided a lower limit of applicability in their model of 100 µg/kg wet weight for fish evaluated in their study, or 2.2 µg/g lipid using the assumed 4.6% lipid content. For wildlife juvenile subyearling salmon at 1% lipid, the lower limit of applicability translates to 22 µg/kg wet weight. The lower limit of applicability is a level below which adverse effects based on mortality, growth or reproduction endpoints were not supported by the data included in the author's review. Some adverse effects, especially those associated with additive effects, are likely to occur below the limit of applicability.
- Since there was no lipid normalizing in these data, it is not possible to determine if there was a lipid effect, and therefore growth effects were scattered.

Importance of lipid values in relation to PCB toxicity for juvenile salmonids

- A number of authors have summarized the importance of lipids to juvenile salmonids in relation to PCB toxicity, and why it is necessary to account for lipid content when calculating effects of PCBs (see Meador et al. 2002 for a review and bullets below for further discussion on importance of lipids).
- Lipids provide a buffering effect which serves as protection from adverse effects of hydrophobic compounds such as PCBs (the higher the lipid content, the higher the resistance because the compounds are not available to induce toxic effects at the site of action in target organs). However, lipid depletion also releases and redistributes stored PCBs to other tissues at rates that depend on blood and nutrient supply and lipid content of specific tissues (summarized by Jørgensen et al. 1999).

- While salmonids have variable lipid content over their lifespan, wild outmigrating subyearlings tend to have very low lipid values (1 to 2 percent) compared to hatchery fish or wild adults (Wood et al. 1960) which makes subyearlings more susceptible to PCB effects during sensitive developmental stages.
- Juvenile salmon deplete lipid reserves in muscle while adapting from fresh to seawater and transforming from parr to smolt (Sheridan et al. 1983), and this smoltification process involves changes in physiological parameters such as gill ATPase, thyroid and pituitary hormones, liver glycogen, blood glucose, and lipid metabolism (Wedemeyer et al. 1980). Stored PCBs in lipids, mobilized and redistributed to various tissues during this process, have been shown to have effects on thyroid hormones (Mayer et al. 1977, Folmar et al. 1982) and affect smoltification.
- Subyearling salmon, with a residence time for some exceeding 50 days in the lower Columbia River estuary (Bottom et al. 2011, McNatt et al. 2016), are likely susceptible to effects on growth and during smoltification when exposed to PCBs during their outmigration. As Meador et al. (2002) noted "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."
- Further, effects on subyearling growth could occur from PCBs. Lundin et al. (2024) reported reduced growth in subyearling salmon exposed to PCBs in the laboratory at environmentally relevant concentrations and postulated that such exposures "…may compromise their ability to achieve sufficient length, mass, or lipid content necessary to survive their first year and beyond."

Diet of juvenile salmonids in lower Columbia River

- Rondorf et al. (1990) reported subyearling chinook salmon primarily fed on zooplankton and terrestrial insects in impoundments and caddisflies & chironomids in free-flowing waters. Moving downriver there was a shift from larger midges and trichopterans to smaller *Daphnia* in response to increased zooplankton density in impounded sections.
- In the lower Columbia River and many other Pacific northwest estuaries, *Corophium* species (amphipods) were the major food item for juvenile salmonids; insects (especially chironomids) were of secondary importance but increased in importance in the upper estuarine areas (Columbia Basin Fish and Wildlife Authority 1996).
- Simenstad et al. (1982) reported the main prey items for juvenile chinook in the lower Columbia River estuary were benthic and epibenthic insects, crustaceans and copepods.
- McCabe et al. (1983) reported *Daphnia* species were important for subyearling chinook salmon in summer in the estuary.
- McCabe et al. (1986) and Durkin and Emmett (1980) reported though subyearlings fed in pelagic areas (where *Daphnia* are abundant), intertidal areas were used more frequently (where large populations of *Corophium* occur). Commonly eaten insect species (e.g., chironomids), are also found in the tidal flats and marsh areas.
- Weitkamp et al. (2022) sampled across 200 km of main channel habitat in lower Columbia River estuary in 2 years (Bonneville Dam to the mouth) where juvenile

salmon were actively feeding and growing; main prey items in stomachs were chironomids and corophiid amphipods. They reported that tidal wetlands were important to both prey and juvenile salmon.

- Bottom et al. (2011) reported that Diptera (flies), *Americorophium salmonis*, and Cladocera (water fleas) had the highest percent contribution to total index of relative importance for subyearlings.

Natural history of corophiid amphipods, important prey items for juvenile salmonids and a potential pathway for contaminants (Note the amphipod *Corophium salmonis* is now *Americorophium salmonis*, though there are other species of *Corophium*)

- *Americorophium salmonis* and other *Corophium* species are tube-dwelling (building their own burrows), 5 mm-long, deposit feeding amphipods found in high densities in fine sediments in shallow bays and shoaling areas in the lower Columbia River estuary (typically in the top 10 cm of sediments).
- The amphipods scavenge and consume diatoms, detritus, sediment, and other items, and can filter feed using the current generated from water filtered through their tubes (Holton et al. 1984, Reish and Barnard 1979).
- *Corophium* species were found to be most common in the upper estuary tidal flats and demersal flats (Simenstad et al. 1984).
- Changing environmental conditions cause *Corophium* males to leave their tubes for the water column (by crawling or actively swimming), rendering them more vulnerable to predation (Columbia Basin Fish and Wildlife Authority 1996).
- The amphipods move upstream or downstream seasonally but generally remain on or near the river bottom (Wilson 1983).
- *Corophium* can be relatively high in lipid content (up to 2.3% lipid in a 1991 sample from Cathlamet Bay from USFWS 2004) whereas lipid in clams and worms used in bioaccumulation tests typically fall between 1 to 2%.
- The strong association of *Corophium* to sediment (compared to other aquatic invertebrates), relatively high lipid content (USFWS 2004), ability to accumulate lipophilic compounds (Vermeer et al. 1993), and high component of the diet for some juvenile salmonids (Bottom et al. 2011) indicates *Corophium* serve as a likely migration pathway for organochlorine contaminants such as PCBs in sediment to expose juvenile salmonids.

Similarity of stomach content concentrations of PCBs in juvenile salmonids to prey

- PCB concentrations in whole body subyearling chinook were similar for salmon collected in the lower Willamette River by Lundin et al. (2021)
- PCBs (sum of 209 congeners) in whole body subyearling chinook collected from the lower Willamette River by the LWG (2006a) were consistently higher than stomach contents, typically by less than half, except at the reference site where whole body was similar to stomach contents in replicate samples.
- In the lower Columbia River, stomach content PCB concentrations were similar and sometimes higher than whole body values in subyearling chinook (Johnson et al. 2013).
- Sherman et al. (2009) collected *Corbicula* clams from five stations in the lower Willamette River and 30 stations in the lower Columbia River from river mile 32

to142. PCB concentrations in *Corbicula* clams closely resembled stomach contents in subyearling salmon (most *Corbicula* values were 30 to 50 µg/kg wet weight (with occasional outliers located a known contaminated sites at over 100 µg/kg) with lipids ranging from 2.4 to 5.6%. In a separate study in the lower Willamette River by the LWG (2006b), percent lipid in 32 composite field-collected *Corbicula* averaged 2.4% (ranging from 1.4 to 4.6%).

- Similarity of PCB concentrations in stomach content and whole body indicate consumption of contaminated prey is the likely exposure route for juvenile salmon (Lundin et al. 2021) and indicates concentrations in invertebrate bioaccumulation test organisms (clams and worms) may be representative of PCBs found in subyearling chinook, and thus results from bioaccumulation testing could be directly compared to salmon TTL values (with lipid normalization).

Uncertainties and Limitations

- All PCBs were measured as Aroclor or Clophen mixtures in studies reviewed by Meador et al. (2002) and Berninger and Tillitt (2019), whereas recent field and laboratory studies totaled PCBs as a select group of congeners (typically 45 used in NOAA studies) and bioaccumulation tests typically measure all congeners. Meador et al. (2002) and Berninger and Tillitt (2019) authors found better correlations between tissue concentrations and effects using Aroclor or Clophen mixture data compared to congener-specific analysis, even though most tissues are currently analyzed and summed as congener-specific PCBs. This rationale is explained in Berninger and Tillitt (2019) supplemental information. It is expected that quantifying congener-specific PCBs in tissues in bioaccumulation test organisms for comparison to the TTL will be associated with some uncertainty but will ultimately result in more reliable data compared to quantifying Aroclors in tissues.
- Toxicity testing on juvenile salmonids consistent with realistic environmental exposure is nearly absent. Though there are studies designed specifically to test juvenile salmonids, they are typically laboratory dietary studies (e.g., Powell et al. 2003, Lundin et al. 2024) with unrealistically high lipid content in the feed (>10%) compared to lipid in the diet of wild juvenile salmon (about <1 to 2.5%).
- Subyearling salmon used in laboratory settings typically have much higher lipid content compared to wild fish (average of 7.1 % lipid in whole body fish in Lundin et al. (2024) which could make the test fish less representative.
- The revised salmon TTL is based on a small selection (eight) of salmon studies from Meador et al. (2002), and eight endpoints are about the minimum recommended to calculate a HC05.
- SSDs require data rules to include and exclude studies, sometimes study selection can be subjective, but the process can help reduce uncertainty compared to relying on a single study to select a TTL.
- Fish collected in the field are exposed to a mixture of compounds, so estimated effect levels in these fish can be confounded by presence of other compounds contributing to toxicity.
- While the "lower limit of applicability" value from the Berninger and Tillitt (2019) review is based on a large database, it is not salmon specific, does not include endpoints tied to effects on smoltification, and therefore may not be protective of

very early life stages of juvenile salmonids. The revised Meador et al. (2002) data are based on salmon-specific studies and include important endpoints related to growth (a key concern for juvenile salmonids in the lower Columbia River) but is limited by a small number of studies. Including sensitive endpoints such as reduction in thyroid hormones (potentially the most important endpoint for transitioning juvenile salmonids) as done in the revised approach provides an important level of protection and confidence into the model.

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