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Donald R. Buhler

A large number of river otter (*Lontra canadensis*) males were collected from areas of high and low contaminant concentrations throughout western Oregon and Washington during the fall and winter of 1994-99. Few significant non-reproductive organ morphometric differences were found in the large series of male otters collected, except for adrenal gland, lung, pancreas, thymus and thyroid gland mass in adult males. With respect to reproductive organ soft tissue mass, Oregon males were significantly heavier. Oregon yearling and adult male otters attain reproductive readiness approximately two weeks earlier than their Washington counterparts, explaining these size differences. Juvenile males from Oregon also appeared to be more developed than Washington juvenile males.

Male river otter hepatic contaminant concentrations exhibited significant regional differences, with males from heavily populated and industrialized regions of western Oregon and western Washington having higher concentrations of polychlorinated aromatic hydrocarbons. Also, otters collected from areas of intensive agriculture had significantly higher organochlorine insecticide and metabolite (OCs) concentrations. Mean liver concentrations of mercury were similar among regions for both juvenile and adult male otters, except the Lower Columbia River males which were significantly lower for both age classes. Although some mean mercury concentrations were elevated (3.6-

13.8 µg g^{-1}, dw), they were well below concentrations considered toxic.

Juvenile male river otters were prepubertal during their first year of life, as signified by the absence of testicular testosterone production and active spermatogenesis. However, testosterone production occurred in yearling male otters when paired testes mass exceeded 11 g, with concurrent spermatogenesis demonstrated. Seminiferous tubule spermatozoa were observed in yearling testes as early as the first week of December.

No morphological reproductive tract abnormalities were noted during necropsy, except for a juvenile male lacking apparent external or internal testes. However, significant inverse relationships were found between hepatic contaminant concentrations (mainly *ortho*-substituted polychlorinated biphenyls [PCBs] and *p,p*'-dichlorodiphenyldichloroethylene [DDE]) and juvenile male gonad mass, testes mass, prostate mass and baculum length and mass, supporting findings from preliminary work. Furthermore, significant inverse relationships were also found between several OCs and PCBs and adult male otter baculum length and mass. The inverse relationships found with adult males implies that the reproductive organ hypoplasia observed in juvenile male otters continues into adulthood as a permanent effect.

Environmental Contaminants in Male River Otters Collected from Oregon and Washington, 1994-99, with Reproductive Organ Hypoplasia Observed in Otter Males

by
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APPROVED:

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Robert Allan Grove, Author

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Environmental Contaminants in Male River Otters Collected from Oregon and Washington, 1994-99, with Reproductive Organ Hypoplasia Observed in Juvenile Males

INTRODUCTION

Thousands of man-made chemicals have been introduced into the environment since the early 1900s, polluting rivers and waterways like those of the Pacific Northwest as a result of agricultural, municipal and industrial activities (van den Berg et al. 2003). Many of these chemical contaminants are persistent polyhalogenated aromatic hydrocarbons known to bioaccumulate and biomagnify as they move through the aquatic food web, effecting species associated with aquatic systems (Mora et al. 1993, Giesy et al. 1995, Heaton et al. 1995, Giesy and Kannan 1998), including humans (Swain 1988, Swain 1991, Jacobson and Jacobson 1996). Long-range global transport of persistent contaminants to regions thought once to be pristine (arctic and antarctic regions), poses ecological concerns on a worldwide scale (Bjerregaard 1995, Voldner and Li 1995). Previous studies in the Columbia River Basin and the Puget Sound have identified and quantified select environmental contaminants of organochlorine insecticides and metabolites (OCs), polychlorinated biphenyls (PCBs), dioxins (PCDDs) and furans (PCDFs) in river otter (*Lontra canadensis*) livers, harbor seal (*Phoca vitulina*) and killer whale (*Orcinus orca*) blubber and skin samples, bald eagle (*Haliaeetus leucocephalus*) eggs, osprey *(Pandion haliaetus*) eggs and fish (Henny et al. 1981, Anthony et al. 1993, Henny et al. 1996, Buck et al. 1999, Buck and Sproul 1999, Kannan et al. 1999, Ross et al. 2000, Simms et al. 2000, Kannan et al. 2002, Henny et al. 2003, Buck et al. 2004, Henny et al. 2004, Ross et al. 2004, Sethajintanin et al. 2004, Grove and Henny 2005, Villeneuve et al. 2005). Contaminants correlated with declines in bald eagle productivity were associated with agricultural pesticides and industrial pollutants (Bowerman 1995, Buck et al. 1999). Pesticides such as p,p'-

dichlorodiphenyltrichloroethane (DDT) and its metabolites caused severe eggshell thinning in bald eagles, double-crested cormorants *(Phalacrocorax auritus*), ospreys, and many other bird species, decreasing productivity and ultimately reducing the size of some bird populations. Mink (*Mustela vison*) are very sensitive to the toxic effects of PCBs, PCDDs and PCDFs (Platonow and Karstad 1973, Aulerich and Ringer 1977, Jensen et al. 1977, Bleavins et al. 1980, Aulerich et al. 1985, Aulerich et al. 1987, Hochstein et al. 1988, Wren 1991, Tillitt et al. 1996, Brunström et al. 2001), adversely impacting the female mink reproductive system and kit survival (Bäcklin and Bergman 1992, Patnode and Curtis 1994, Restum et al. 1998). Mink numbers along the Lower Columbia River of Oregon and Washington have declined severely over the past 20 years, which may be due to these persistent organic pollutants (Henny et al. 1981, Henny et al. 1996).

A preliminary contaminant study was conducted during the fall and winter of 1994-1995 using frozen river otter carcasses collected by trappers in both the Portland, Oregon metropolitan area and at various sites downstream along a 120 mile stretch of the Lower Columbia River (Henny et al. 1996). Baculum and testes size of juvenile river otters from the Lower Columbia River were negatively correlated with increasing concentrations of several halogenated contaminants (Henny et al. 1996). None of the other body parameters examined were negatively correlated with the contaminants studied. Furthermore, reproductive organs of adult male river otters collected on the Lower Columbia River did not appear to be significantly affected by increasing contaminant concentrations. The observed reproductive organ hypoplasia of juvenile male river otters was correlated with 6 different OCs, 29 PCBs, 2 PCDDs, and 4 PCDFs. Small sample size and numerous contaminant correlations resulting from the complex mixture of chemicals released from the heavily industrialized Portland area and upstream sites, made it difficult to identify the specific contaminant(s) responsible for the observed hypoplasia encountered with juvenile male otters. However, several of these contaminants are known endocrine-disrupting

chemicals which may be responsible for the observed reproductive organ hypoplasia in juvenile male otters, and continued study of these effects could provide useful information concerning both wildlife and human health risk.

Endocrine Disruption

Survival and reproductive success of an organism requires an ability to adapt to constantly changing external environmental conditions, while maintaining a constant internal environment within very narrow tolerances (Goodman 2003). This maintenance or homeostasis of an organism is done through chemical signaling between cells within a tissue, organ and at a distance in order to coordinate the cellular activities of the various tissues and organs within an organism. Communication between cells by chemical signals called hormones can be done through direct contact (autocrine), over short distances by simple diffusion (paracrine) or by entering the circulatory system (endocrine). Some cells can even respond to their own chemical signals (cytocrine). A hormone is defined as a chemical substance that is released into circulation in small amounts that, after delivery to its target cells, elicits a typical physiological response. Hormone concentrations entering into the circulatory system via simple diffusion are diluted, and achieving meaningful concentrations of 10^{-7} to 10^{-10} molar requires the coordinated secretion by a mass of cells within an endocrine gland (Goodman 2003). Therefore, the primary role of the endocrine system is to coordinate the dynamic response and feedback of distant target tissues from signals of another organ or tissue (WHO 2002).

Sex hormones, by nature of their bio-active potency, are crucial in the reproductive process by precisely controlling development, puberty, behavior, gametogenesis, and integrated sexual function (Chapin et al. 1996). Androgens are male sex steroids secreted from the testes, and to some extent the adrenals, that play a critical role in the development, differentiation and maintenance of the male reproductive system. The male reproductive system consists of a diverse number of physiological functions that mediate signals from the brain, promote

cell proliferation and differentiation, to the initiation of apoptosis of unnecessary cells, and all are influenced to some extent by steroid hormones. For example, testosterone (T) and 5α-dihydrotestosterone (DHT) controls the development, differentiation and function of the male reproductive system. Other organs (i.e., brain, hair, muscle, skeleton, skin) are also under the influence of these androgens. However, these molecular mechanisms of androgenic modulation have not been well characterized (Walker 2003).

The World Health Organization (WHO 2002) defined an endocrine-disrupting chemical (EDC) as "an exogenous substance or mixture that alters endocrine function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny and/or (sub) populations." Specifically, EDCs can alter hormone synthesis, secretion, transport, binding, action or elimination of endogenous hormones responsible for the maintenance of homeostasis, immune function, reproduction, development and/or behavior such that endocrine regulatory function is adversely impacted. The subject of endocrine disruption is not a new topic, but has come to the forefront over the last 30 years due to reports of altered reproductive development in wildlife (Fry and Toone 1981, Fox 1992, Colborn et al. 1993, Guillette et al. 1995, Guillette and Crain 1996, Guillette et al. 1999, Vos et al. 2000, Willingham et al. 2000, Hayes et al. 2002, Hayes et al. 2003, Oehlmann and Schulte-Oehlmann 2003, Feist et al. 2005); reports of increased incidence of human male reproductive tract abnormalities (Jirasek 1971, Leung et al. 1985, Fisher 2004a, Swan et al. 2005); and reports of decreased adult human sperm counts in some regions of the world (Nelson and Bunge 1974, Leto and Frensilli 1981, Bostofte et al. 1983, Carlsen et al. 1992, Skakkebaek and Keiding 1994, Auger et al. 1995, Jensen et al. 2002). Several halogenated and non-halogenated hydrocarbon chemical families have been implicated as EDCs. Field and laboratory studies have shown that exposure to certain of these chemicals have resulted in adverse effects of some wildlife species and populations (i.e., *p,p*'-dichlorodiphenyl-dichloroethylene [DDE] related eggshell thinning in bald eagles). Effects from

EDC exposure can vary from subtle physiological and behavioral effects to permanently altered sexual differentiation. This is especially true for mammals exposed to EDCs during the critical periods of reproductive organ development and differentiation (both pre- and post-natally) in which the young are exposed by both *in utero* and lactational routes (Mably et al. 1992b, Mes et al.1995). Aquatic species at the top of the food chain seem to be most affected by EDCs (Colborn and Thayer 2000), though effects have been reported in terrestrial species as well. Most research concerning EDCs have been focused on the female reproductive system, however, recent studies have shown that the male reproduction system can be altered by certain of these chemicals as well.

EDCs can affect the endocrine system of an organism by: 1) binding and activating a hormone receptor (agonistic), thus mimicking the natural ligand; 2) binding, but blocking hormone receptor activation or interfering with its function (antagonistic); 3) binding to other receptor types whose expression would conflict with the expression of the receptor of concern (i.e., sex steroid receptors); 4) altering hormone receptor numbers which would effect cellular hormonal response; 5) altering hormone interaction with respective circulatory carrier proteins, which alters hormone availability; 6) inducing or inhibiting hormone synthesis or metabolism, thereby perturbing normal hormone levels and 7) direct toxicity of target endocrine cells. However, specific mechanisms of action from EDC exposure in most studies reported, are poorly understood, and it is hard to differentiate between primary and secondary exposure routes and/or effects (WHO 2002). Though receptor mediated mechanisms have been on the forefront of endocrine disruptor research, the other mechanisms mentioned (hormone synthesis, transport, metabolism, clearance) have shown equal importance (Hayes et al. 2002, WHO 2002, Hayes et al. 2003).

Evidence of EDCs in Wildlife

The majority of knowledge pertaining to the exposure effects of EDCs in mammalian endocrine systems has come from *in vivo* and *in vitro* animal

laboratory studies. Though there is sufficient evidence that mammalian wildlife is affected by environmental contaminants, only limited evidence exists to support the contention that these effects are a result of endocrine dependent mechanisms (WHO 2002). However, there are several aspects of vertebrate endocrinology, such as key hormonally regulated events in reproductive development and differentiation, that are very similar between wildlife species and laboratory animal models or humans (WHO 2002). To date, only a few wildlife species have been shown to be more sensitive to EDCs (i.e., mink, bald eagle) than laboratory animal models or humans, and conclusions on general wildlife sensitivity to EDCs are often hindered by large data gaps of species specific biology. It is also important to note that the endocrine systems of invertebrate wildlife can also be adversely impacted by EDC exposure. Life history differences between wildlife species also tend to complicate efforts to understand potential EDC related effects across species lines. Therefore, special consideration must be made of each species unique biology that might make them susceptible to the potential effects of EDCs (i.e., delayed implantation, oviparous reproduction). For example, mammals are represented by over 4,500 species, possessing a wide array of life history strategies, from the modern eutherian or placental mammals to the more primitive metatherian mammals (marsupials) like the opossum (*Didelphis virginianus*) which give birth to highly underdeveloped young which finish a prolonged lactational development inside of a pouch, to the even more primitive egg laying monotreme mammals like the duck-billed platypus (*Ornithorhynchus anatinus*).

Feeding strategies directly impact exposure to EDCs as evidenced by contaminant body burdens in rodents, where insectivores (shrews) had the highest contaminant body burdens, followed by omnivores (cricetid mice) and finally herbivores (voles) (Talmage and Walton 1991). Mammal groups like mustelids (otters, mink), pinnipeds (seals, sea lions) and cetaceans (dolphins, whales) reside at the top of the food chain and are particularly vulnerable to EDCs as species within these groups accumulate large body burdens of these

highly persistent contaminants through biomagnification from contaminated prey (WHO 2002).

A number of reproductive effects in aquatic and terrestrial mammalian wildlife species have been associated with contaminants. High concentrations of PCBs have been reported in the Eurasian otter *(Lutra lutra*) and mink, which have been associated with reproductive effects/population decline and decreased litter size/kit survival, respectively (Heaton 1991, Wren 1991, Kihlström et al. 1992, Patnode and Curtis 1994, Smit et al. 1994, Restum et al. 1998, Brunström et al. 2001). Sterility, implantation failure, decreased fecundity and premature pupping have been associated with OCs and PCBs in seals and sea lions (Delong et al. 1973, Helle 1980, Reijnders 1986). It has been proposed that reproductive impairment and low kit survival in mink (and possibly other mammalian species) are caused by arylhydrocarbon receptor (AhR) agonists like TCDD and PCBs, though no specific mechanism has been elucidated (Brunström et al. 2001). Reduced testosterone concentrations and fecundity, and hermaphroditism observed in porpoises and whales have been associated with OCs, PCBs and TCDD (Subramanian et al. 1987, Martineau et al. 1988, De Guise et al. 1994). Lower thyroid hormone levels were associated with PCB concentrations in harbor seals (*Phoca vitulina*) (Brouwer et al. 1989, Brouwer et al. 1998). In all of the incidences mentioned above, few were associated with proposed EDC pathways and the etiologies noted remain unsolved.

Reproductive effects have been seen in several species of birds, beginning with DDE related eggshell thinning. Reproductive failure due to DDE-induced eggshell thinning and crushed eggs has been well documented over the years (Ratcliffe 1967, Peakall et al. 1973, Henny and Herron 1989, Bignert et al. 1995). However, the DDE associated eggshell thinning was only recently elucidated (Lundholm 1997). DDE (p,p'-DDE specifically) inhibits prostaglandin synthase, reducing prostaglandin E2 in the eggshell gland mucosa, which reduces calcium transport across the mucosa into the eggshell gland lumen.

The feminization of avian males has been documented and associated with a number of contaminants. Persistent Müllerian ducts, ovotestes and female-female pairing during nesting have been associated with the DDTs and methoxychlor (Fry and Toone 1981, Fry 1995). Embryonated gull eggs injected with environmentally relevant concentrations of *o,p'*-DDT, *p,p'*-DDE and methoxychlor resulted in malformed feminized male sex organs (Fry and Toone 1981). The OCs were thought to induce ovary like primordial germ cells within the left testes, along with inducing retention of one or both Müllerian ducts. Females collected from Puget Sound glaucous-winged gull (*Larus glaucescens*) colonies in 1984 exhibited eggshell thinning and retained right oviducts, which under normal conditions completely regress (Fry et al. 1987). Major contaminants within the Puget Sound were heavy metals, PCBs and polyaromatic hydrocarbons (PAHs). Decreased hatching and reproductive failure has been also associated with OCs, PAHs, PCBs, TCDDs and TCDFs (Kubiak et al. 1989, Giesy et al. 1994).

Reproductive effects in aquatic reptiles and amphibians have been associated with several contaminants (Vos et al. 2000). The crocodilian family, several turtle species and some lizard species, do not have distinct sex chromosomes which determine gender (Crain and Guillette 1998). Rather, sex is determined during organogenesis by incubation temperature (temperature-dependent sex determination, TSD). However, reptiles having TSD to determine sex are developmentally sensitive to exogenous hormones and EDCs which can alter temperature-dependent sex ratios. Administration of E2 during the temperature sensitive period of sexual differentiation produced females at male producing temperatures (Crain and Guillette 1998). Red-eared slider turtles (*Trachemys scripta elegans*) exhibiting TSD show sex reversal following embryonic exposure of hydroxylated PCBs in experimental studies, significantly altered sex ratios, producing more females as did E2 treated eggs (Bergeron et al. 1994). Snapping turtles (*Chelydra serpentinia serpentinia*) from OC contaminated sites near Ontario, Canada showed a higher of incidence of male

feminization when compared to reference sites (De Solla et al. 1998). Perhaps the best documented case is that of American alligators (*Alligator mississippiensis*) living near contaminated Lake Apopka. A major pesticide spill containing primarily dicofol, but also containing DDT, reduced the juvenile population of alligators by 90% immediately following the spill as a result of poor egg viability and offspring survival (Woodward et al. 1993, Guillette et al. 1994). Alligator eggs contained a variety of pesticides and their metabolites, including DDD, DDE, dieldrin, trans-nonachlor, oxychlordane and toxaphene, with DDE the major contaminant. Developmental abnormalities observed in alligator hatchlings included abnormal gonad morphology, altered steroidogenesis and altered sex steroid concentrations in males and females when compared to reference animals (Guillette et al. 1995). Neonatal female alligators collected from Lake Apopka had prominent polyovular follicles and multinucleated oocytes, which were not observed in females from reference sites. Six month old male alligators had poorly organized gonads with germ cells undergoing premature spermatogenesis and small phalli (Guillette et al. 1994). Altered plasma steroid concentrations correlated with abnormal gonad morphology and small penis size. To date, however, the exact causative agents and mechanisms responsible for the endocrine disruption are still unknown, though DDE was the principal contaminant. The endocrine effects noted may result from EDCs interacting with the estrogen and/or progesterone receptors, which is supported by receptor competition assays (Voiner et al. 1996). Androgens are also essential for the normal development of the male reptile reproductive system (Guillette et al. 1994). DDE is known as an antiandrogenic chemical (Kelce et al. 1995), though there are no data available that indicates DDE binds to the reptilian androgen receptor. However, it is possible that DDE could act as an AR antagonist in developing male alligators.

Laboratory studies indicate that DDT may be estrogenic in other reptilian and amphibian species (Palmer and Palmer 1995). Dosing with *o,p*-DDT induced the estrogen controlled hepatic synthesis of the female precursor yolk

protein vitellogenin in male red-eared slider turtles (*Trachemys scripta*) and African clawed frogs (*Xenopus Laevis*). Dieldrin and toxaphene also induced vitellogenin production in the male clawed frogs.

Recently, atrazine was shown to induce gonad dysgenesis and hermaphroditism in male African clawed frogs and leopard frogs (*Rana pipiens*) at environmentally relevant concentrations (Tavera-Mendoza et al. 2002, Hayes et al. 2003). Several studies suggest that atrazine induces cytochrome P450 aromatase (CYP19), converting T to E2, thus feminizing the male frogs (Sanderson et al. 2001, Keller and McClellan-Green 2004).

Probably the best example of xenoestrogens in the aquatic environment involves fish exposure to sewage effluents (Sumpter 1995, Vos et al. 2000). Exposure of male fish to domestic sewage discharges induces the synthesis of the female egg protein vitellogenin, with feminization of the male reproductive system also noted. Many natural and man-made chemicals known to be estrogenic to fish are found in sewage effluents.

Finally, imposex has been found in invertebrate gastropods and bivalves exposed to tributyltin (TBT). The first reported case of imposex was in 1970, involving the invertebrate species *Nucella lapillus* (Blaber 1970), with the term imposex coined by Smith (1971). Imposex is the imposition of male sex organs onto females, including the penis and vas deferens (WHO 2002). The frequency of imposex and degree of penis development are related to the concentration and duration of TBT exposure. The consequences for many invertebrate species exposed to TBT are sterility, distortion of sex ratios, juvenile recruitment reductions and reduced population size. About 150 prosobranch gastropod species worldwide have been affected by exposure to TBT and other organotins (Matthiessen et al. 1999). Imposex is androgen driven as imposex induction can be blocked by antiandrogens, which points to androgen receptor involvement (Santos et al. 2005). It has also been hypothesized that the retinoid X receptor may be involved as TBT has a strong affinity for the receptor. The natural ligand 9-cis retinoic acid can also induce imposex above physiological concentrations.

In summary, many wildlife species are being exposed to biologically active concentrations of EDCs, including natural products, OCs, PCBs, TCDDs, TCDFs, organotins, alkylphenols, phthalates and other chemicals (WHO 2002, Vos et al. 2000). Impaired reproduction and development have been causally linked to EDCs in many species of several taxa, including mammals, birds, reptiles, amphibians, fish and invertebrates. Effects observed as a result of EDC exposure range from subtle changes in physiology and behavior, to permanent structural and functional alterations, including altered sex differentiation with malformed reproductive organs, altered behavior and compromised immune function. Environmental toxicology studies are complicated by a variety of factors that may impact an animal's basic welfare, like habitat loss, human disturbance, food availability and disease state. Other confounding factors are related to our limited knowledge about individual aspects of endocrine, reproductive and developmental biology for the majority of wildlife studied. But, wildlife field studies also provide clues which can be pursued under controlled laboratory conditions. Given the responses observed in wildlife so far, it is important to continue environmental research to further elucidate observed EDC effects on wildlife populations.

Current Mammalian Male Reproductive Endocrinology and Proposed Mechanisms of Endocrine Disruption

Receptor mediated binding of endogenous hormones to their respective receptors occurs at the level of the cell surface, cytoplasm or nucleus, invoking a complex series of events that alters gene expression characteristic of the specific hormone (Birnbaum 1994, Fox 2004). Normal hormonal regulation of gene expression is critical in modulating appropriate biological function, including cellular proliferation and differentiation during development. In the case of male sex steroids, the principle action of the androgens T and DHT is to regulate gene expression through the androgen receptor (AR) which belongs to the superfamily of nuclear receptors. Nuclear receptors are ligand-inducible transcription factors

that mediate the signals of a variety of lipophilic hormones (Truss and Beato 1993, Beato and Klug 2000). The AR can regulate gene expression directly by interacting with specific elements in the regulatory regions of target genes (Riegmen et al. 1991), or indirectly by activating growth factor signaling pathways (Peterziel et al. 1999). As with other members of the nuclear receptor family, the AR has 4 major functional regions that are responsible for transcriptional activity: the N-terminal transactivation domain (TAD), a central DNA-binding domain (DBD), a C-terminal ligand binding domain (LBD) and a hinge region that connects the central binding domain to the C-terminal ligand binding domain (Mangelsdorf et al. 1995). The androgen receptor DBD exhibits a high degree of amino acid homology with other members of the glucocorticoid receptor subfamily (GR), the progesterone receptor (PR) and the mineralocorticoid receptor (MR). As a result, these receptors recognize similar, if not identical, hormone response elements. Ligand binding of the hormone to the AR initiates several events, allowing the regulation of target genes by the receptor (Figure 1). An occupied receptor undergoes an allosteric change of the LBD which dissociates the receptor from heat shock proteins (hsp 70 and 90), and dimerizes with another AR-hormone complex. The dimerized AR pair then translocates to the nucleus, recognizes and binds to the androgen response element (ARE), recruiting coactivators, repressors and transcription regulators, forming a pre-initiation complex that interacts with the basal transcription machinery which results in the initiation of transcription (Roy et al. 2001). It has been believed that mammals possessed a single AR, as a single base substitution of the receptor causes complete phenotypic sex reversal as evidenced in humans with androgen insufficiency syndrome (Quigley et al. 1995). However, recent studies have identified two isoforms of the AR, in which AR-A can antagonize the transcriptional activity of AR-B (Liegibel et al. 2003, Gao and McPhaul 2006, Gatson et al. 2006). The apparent modulatory mechanism of AR-A may be relevant to activation/inhibition of signaling pathways and gene transcription regulation and how EDCs interact with the two

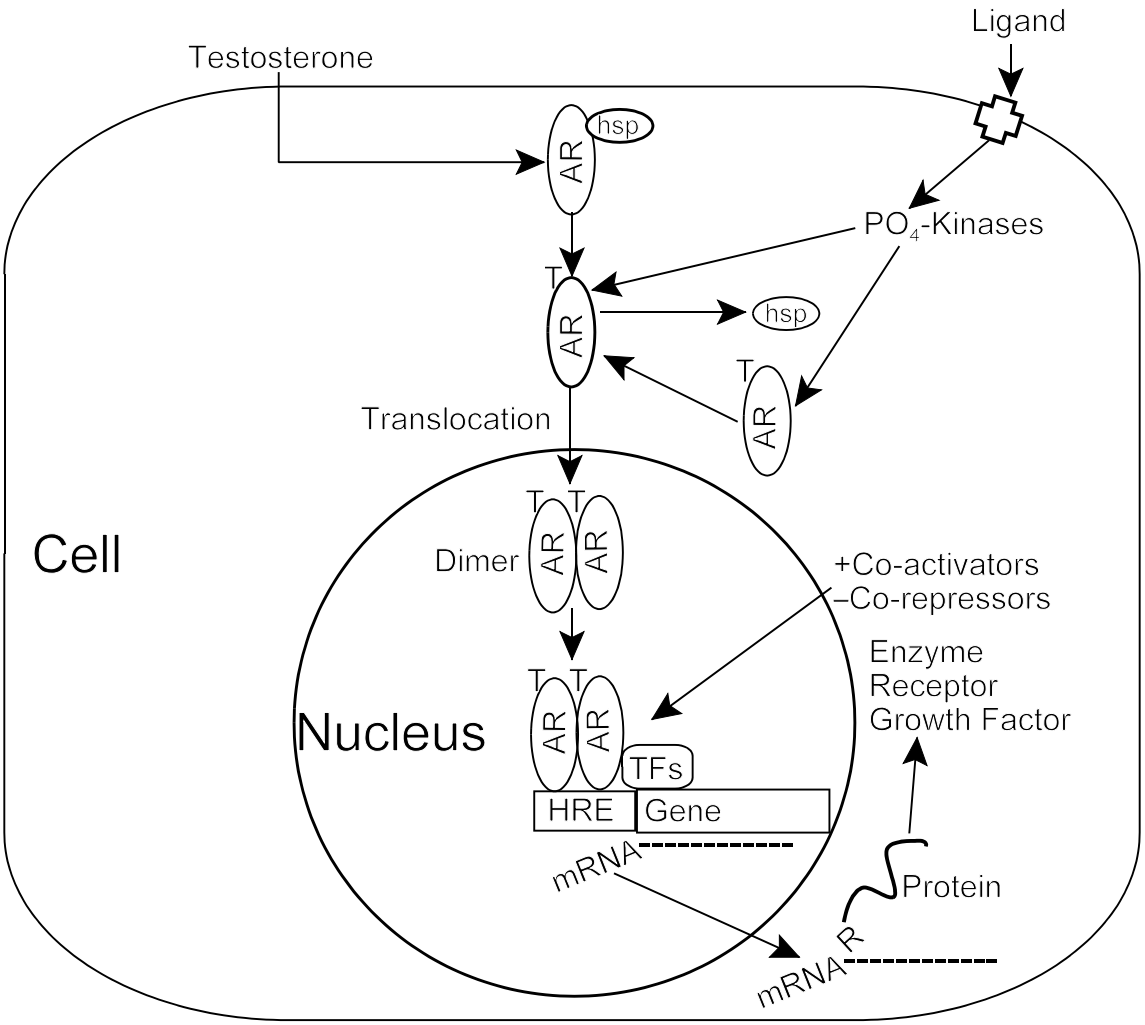


Figure 1. Simplified diagram of an androgen receptor-mediated event that is prerequisite to normal androgen induced protein production.

receptors.

Several EDCs bind to the AR and are considered antiandrogenic (antagonistic), such as the metabolites of vinclozolin 2-[[(3.5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid (M1) and 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2), hydroxyflutamide, DDE and phthalates. These chemicals antagonize androgen binding to the AR (Gray et al. 1994, Kelce et al. 1994, Kelce et al. 1995, Sharpe et al. 1995, Kelce et al. 1997, Gray 1998, Mylchreest et al. 1998, Mylchreest et al. 1999, Ostby et al. 1999, Wolf et al. 1999, Mylchreest et al. 2000, Gray et al. 2001, Sharpe 2001, Sultan et al. 2001,

Farla et al. 2005, Kavlock and Cummings 2005). Androgen receptor antagonists, in general, still induce the translocation of the AR receptor to the androgen response element (ARE) on DNA, but inhibits binding to the ARE. This blocks AR-induced transactivation. Early *in utero* exposure to these types of EDCs inhibits the sexual differentiation of male embryos, which is manifested by hypospadias, cleft phallus, epididymal and prostate agenesis, ectopic undescended testes and reduced anogenital distance at birth, with abnormal sexual behavior and spermatogenesis at puberty (Gray 1998). In fact, recent evidence suggests that the metabolites of vinclozolin may have long-term transgenerational effects on male rat reproductive development and fertility, which may be associated with altered DNA methylation of the male germ cell line (Anway et al. 2005). Little is known about antagonistic disruption of AR mediated second messenger pathways of DNA expression regulation.

Cell surface signaling pathways also use the AR to initiate cellular response in the presence of the endogenous hormone ligand. Cell surface mediated responses by steroid hormones have been described in at least 10 cell types (Sun et al. 2003, Walker 2003, Gatson et al. 2006). Some cell types use surface receptors to transduce hormone signals, initiating final cellular characterization. Hormone receptor mediated initiation of second messenger systems activate signal transduction pathways involving cyclic adenosine monophosphate (cAMP), inositol 1,4,5-triphosphate (IP3), phospholipase C, diacylglycerol (DAG) and calcium ions (Figure 2). Each type of second messenger system is capable of activating multiple kinases such as the MAP kinase cascade which is regulated by hormones and is important in altering the activity of transcription factors (gene expression regulation). Also, intracellular cross-talk by agonist-induced receptor changes can modulate steroid-induced transcription (Klinge et al. 1999, Falkenstein et al. 2000). Intracellular cross-talk may even occur without a steroid ligand (Figure 3). Epidermal growth factor may be able to activate estrogen receptor α (ERα) through the MAP kinase pathway by directly phosphorylating the critical serine 118 of the receptor (Bunone et al. 1996).

Nongenomic steroid effects are characterized by their rapid onset of action (seconds to minutes) and their insensitivity to inhibitors of transcription and protein synthesis (Falkenstein et al. 2000, Walker 2003). These rapid effects are probably mediated through receptors with distinct pharmacological properties. Nongenomic response is usually characterized as being direct where the steroid acts alone as the agonist in direct action, or indirectly by needing a partner agonist to generate the response (Falkenstein et al. 2000, Walker 2003). Signaling of nongenomic steroid action is thought to operate through nonspecific binding without receptor involvement, specific binding through the classical steroid receptor, or specific binding through a non-classical steroid receptor. Nongenomic responses seem to be specific and depend on the type of steroid, cells, tissues or species involved (Falkenstein et al. 2000, Walker 2003). However, these rapid signaling pathways share homologies with traditional second messenger systems (i.e., $[Ca^{2+}]$, cAMP, MAP kinase). For example, Sertoli cells in the seminiferous tubules of the testes are responsible for the maintenance of development and differentiation of spermatogonium to mature spermatozoa. Sertoli cells surround developing germ cells, forming special occluding tight junctions which prevents direct transport of factors larger than one kilo Dalton (kDa) to developing germ cells beyond the spermatogonium stage. The Sertoli cells provide a protecting microclimate for developing germ cells, but this action requires the Sertoli cells to provide growth factors and nutrition needed by the developing germ cells. Sertoli cells are in turn controlled by T produced by the Leydig cells and follicle-stimulating hormone (FSH) produced by the anterior pituitary. The hormonal signals (transcriptional) act exclusively on the Sertoli cells to produce the required factors for the developing germ cells. Follicle-stimulating hormone is an important facilitator of spermatogenesis, but does not appear to be essential for maintenance of germ cell development (Plant and Marshall 2001). However, T is essential for the maintenance of germ cell development. A relatively high concentration of T (70 nM) must be present if spermatogenesis is to occur in humans. The

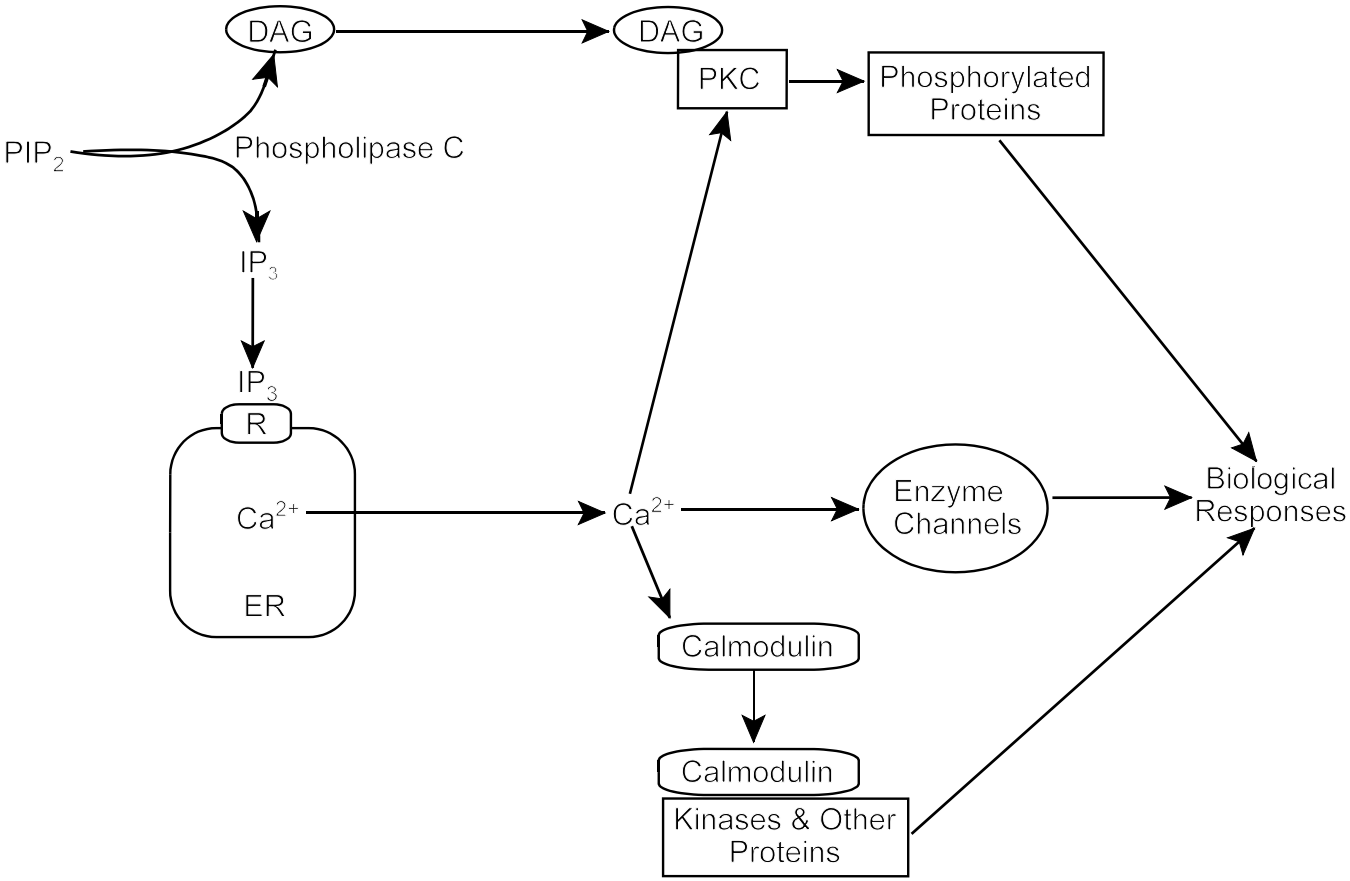


Figure 2. Signal transduction through the inositol triphosphate (IP_3) and diacylglycerol (DAG) second messenger system. Phosphatidylinositol 4,5-biphosphate (PIP_2) is cleaved into IP_3 and DAG by phospholipase C. DAG activates protein kinase C (PKC) which phosphorylates other proteins to produce cell-specific effects. IP_3 binds to a receptor on the endoplasmic reticulum, releasing calcium (Ca^{2+}) which further activates PKC, activates or inhibits enzymes or ion channels or binds to calmodulin which binds to and activates protein kinases and other proteins (Goodman 2003).

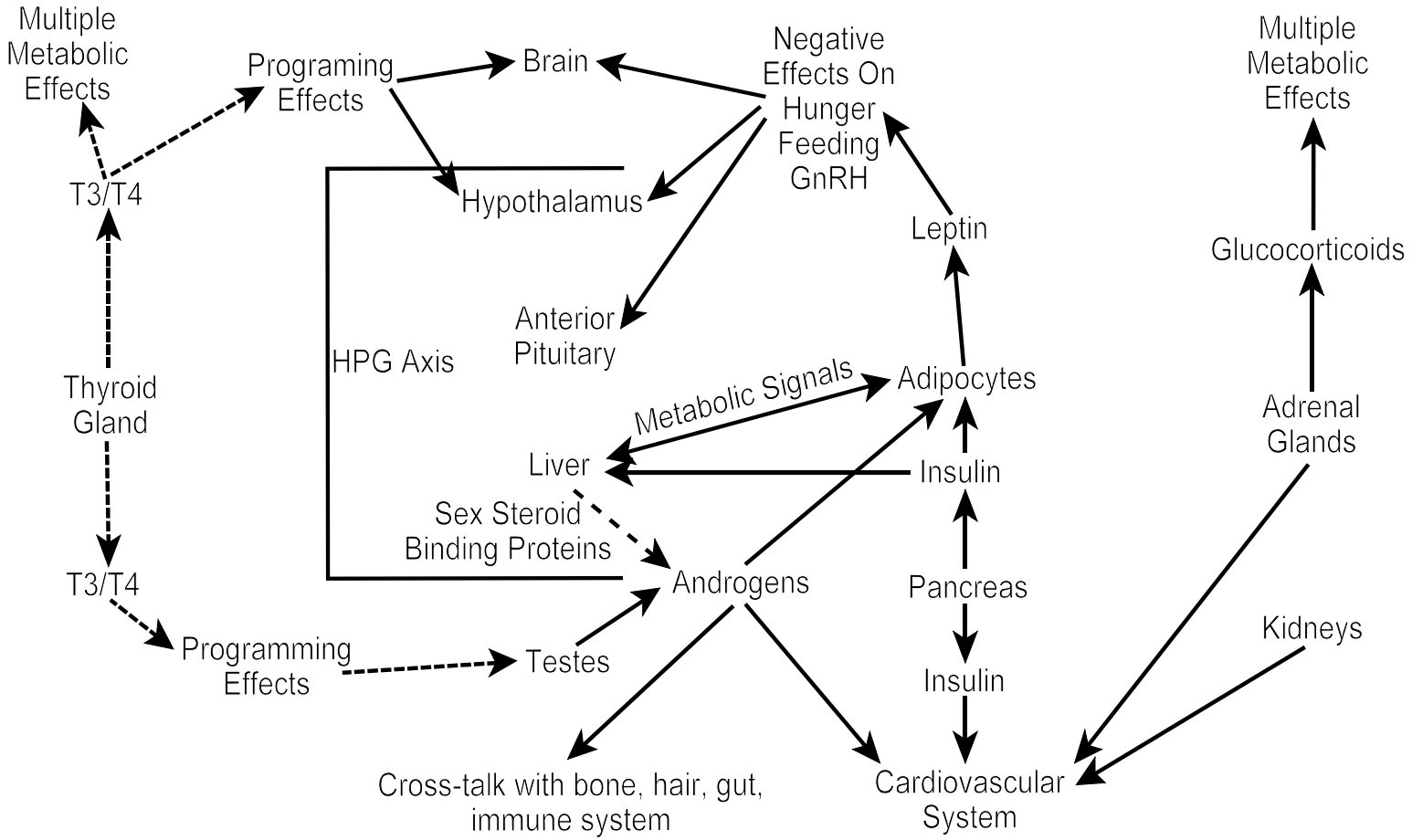


Figure 3. Diagram illustrating some of the cross-talk which occurs between the mammalian hypothalamus-pituitary-gonadal (HPG) axis and other endocrine axies of the body. It is important to note that each endocrine axis interacts at multiple levels with the other endocrine axes, integrating physiological functions.

paradox though, is that only 1 nM of T is required to saturate AR binding to the corresponding response elements (Sharpe 1994). Also, calcium ion levels in Sertoli cells are elevated only seconds after androgen stimulation, the response which cannot be associated to the AR-DNA interaction and initiation of gene expression (Gorczynska and Handelsman 1995, Lyng et al. 2000). These two observations suggest that the action of T on Sertoli cells are through genomic and nongenomic pathways.

Octylphenol, nonylphenol and bisphenol A are examples of known potent inhibitors of both non-genomic Ca^{2+}-ATPase activity and Ca^{2+}-uptake in rat testis microsomes (Hughes et al. 2000, Kirk et al. 2003). Inhibition of the Ca^{2+} pumps in the testis endoplasmic reticulum by these chemicals disrupts Ca^{2+} homeostasis in rat Sertoli cells. The resultant sustained increase in Ca^{2+} concentration decreases Sertoli cells numbers through Ca^{2+} dependent apoptosis, which ultimately decreases the number of sperm that can be produced (Hughes et al. 2000). Endocrine disruption by phthalates may also interact through similar if not the same non-genomic pathways.

Endocrine disruption is not limited to those exogenous agents directly interacting with hormone receptors. EDCs can inhibit hormone synthesis, induce hormone metabolism, interfere with hormone transport, interfere with the feedback systems of hormone regulation or by overt toxicity (WHO 2002, Fisher 2004b). At the onset of puberty, T production is controlled through a classical yet very complicated negative feedback loop system (Figure 4) involving both the hypothalamus and the anterior pituitary (McCann et al. 2002). In general, the hypothalamus releases a short lived 10 amino acid peptide neurohormone(s) called gonadotropin-releasing hormone (GnRH) into the hypophysial portal system (HPS). Blood moving through the HPS arrives at the anterior pituitary (AP), where GnRH induces the production and secretion of the gonadotropins luteinizing hormone (LH) and FSH from specialized gonadotrophic cells of the AP. Recent evidence, however, suggests that GnRH is in fact two releasing hormone factors of luteinizing hormone-releasing-hormone (LHRH) and follicle-

stimulating hormone-releasing-hormone (FSHRH), because the pulsatile release of LH and FSH can be dissociated (McCann et al. 2002). Luteinizing hormone and FSH are heterodimeric glycoproteins consisting of the same alpha chain (which is also found in thyroid stimulating hormone) but having different beta chains of 115 amino acids, giving each glycoprotein their distinct hormonal properties and allowing each hormone to bind with its respective receptor. Luteinizing hormone acts on the interstitial Leydig cells to induce testosterone synthesis, while FSH interacts with the Sertoli cells to promote and maintain sperm production. Testosterone in turn inhibits GnRH synthesis at the level of the hypothalamus and the anterior pituitary when T plasma levels are high enough. Inhibin is a protein hormone produced by the Sertoli cells which inhibits FSH synthesis at the anterior pituitary (Burger and Robertson 1997, Goodman 2003). Inhibin is synthesized in direct proportion to the number of sperm produced, and is a controlling factor in the number of sperm produced. If sperm production is large, then inhibin synthesis and secretion is increased and FSH synthesis and release is decreased. If sperm production is low, then inhibin production is low and FSH synthesis is high. Activin is produced in the testes and the AP, stimulating production of FSH via the hypothalamus, and is further regulated by the binding protein follistatin (Anderson et al. 1998, Calogero et al. 1998). Intra-pituitary regulation (autocrine) of gonadotrophs by inhibin and activin has also been suggested from findings with male rodents and primates (Winters and Moore 2004). Müllerian inhibiting hormone (MIH) may also play a role as an auto-regulatory loop by increasing FSH expression in the pituitary, while MIH expression is decreased by FSH in the gonad (Bédécarrats et al. 2003).

PCB exposure was found to alter pituitary LH secretion in response to hypothalamus GnRH stimulation in rats (Desaulniers et al. 1999, Khan and Thomas 2001, Gore et al. 2002, Desotelle et al. 2005, Yamamoto et al. 2005). PCBs inhibit GnRH-stimulated LH secretion by altering GnRH gene expression with a concurrent decrease in GnRH production and secretion.

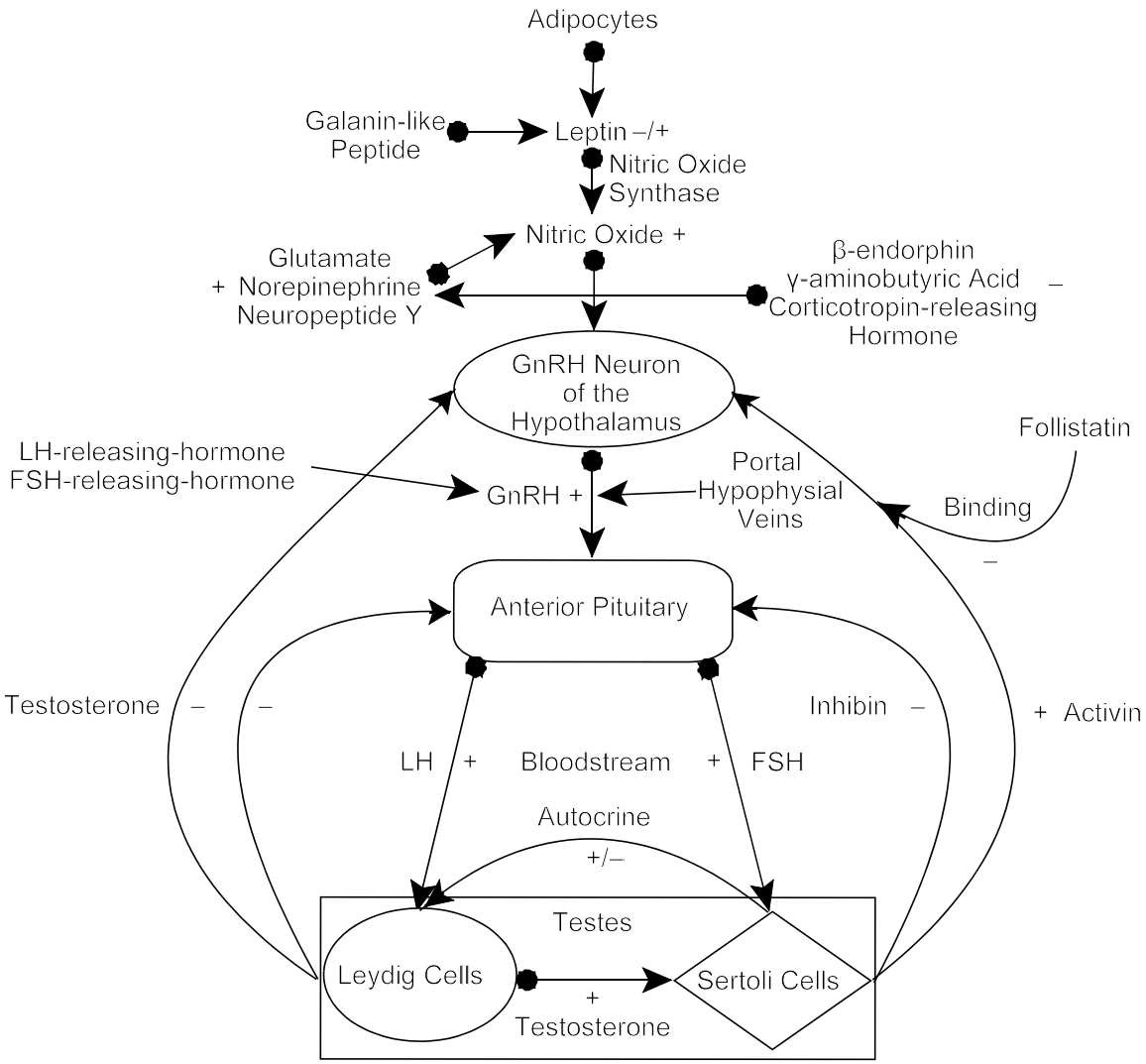


Figure 4. Diagram of the hypothalamic-pituitary-gonadal axis regulatory feedback pathways for the secretion and action of Gonadotropin-releasing Hormone (GnRH) , Luteinizing Horomone (LH) and Follicle-stimulating Hormone (FSH).

Nitric oxide (NO) is a gaseous signaling molecule that has been shown to exert multiple effects on several tissue types, including the brain (Knauf et al. 2001). Nitric oxide has been implicated in several reproductive mechanisms and behaviors, including hormone synthesis, lordosis, ovulation, and penile erection. The NO molecule is also involved in the control of GnRH/LH release at the level of the hypothalamus by modulating GnRH neuronal activity (Bhat et al. 1998, Rettori and McCann 1998, Dhandapani and Brann 2000, Knauf et al. 2001). The gaseous molecule stimulates the release of GnRH at the terminal end of the GnRH neurons located in the median eminence of the brain. Nitric oxide (because of its potency) is thought to play a major role in the control of the GnRH neuroendocrine axis, thus controlling reproductive functions (Knauf et al. 2001). Nitric oxide modulation is also found at the level of the AP (Figure 5) by activating guanylyl cyclase, converting guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) which activates protein kinase G. Recent data indicate that FSHRH acts on its specific receptor via a calcium-dependent NO pathway to release only FSH, while LHRH causes the release of LH with some release of FSH (McCann et al. 2002). Leptin (a 167-amino acid protein) also plays a part in modulating LH and FSH synthesis through the NO pathway at both the hypothalamus and the AP and may also be integral in the induction of puberty (De Biasi et al. 2001, Woller et al. 2001, McCann et al. 2002, Watanobe 2002, Ponzo et al. 2004, Reynoso et al. 2004). The leptin protein is produced by adipocytes and is thought to be a metabolic regulator of reproductive capability based on nutritional status. Leptin effects in the hypothalamus may be mediated by a galanin-like peptide produced in the hypothalamus (Seth et al. 2004) and by thyroid hormones (Costa da Veiga et al. 2004). Finally, NO is also involved as a signaling molecule for the production, capacitation and acrosome reaction of spermatozoa (Herrero et al. 2003). No adverse effects on NO regulatory system as a result of EDC exposure have been reported to date.

There appears to be an endless variety of inputs to hypothalamic GnRH neurons, where GnRH cells are able to recognize and respond to virtually every

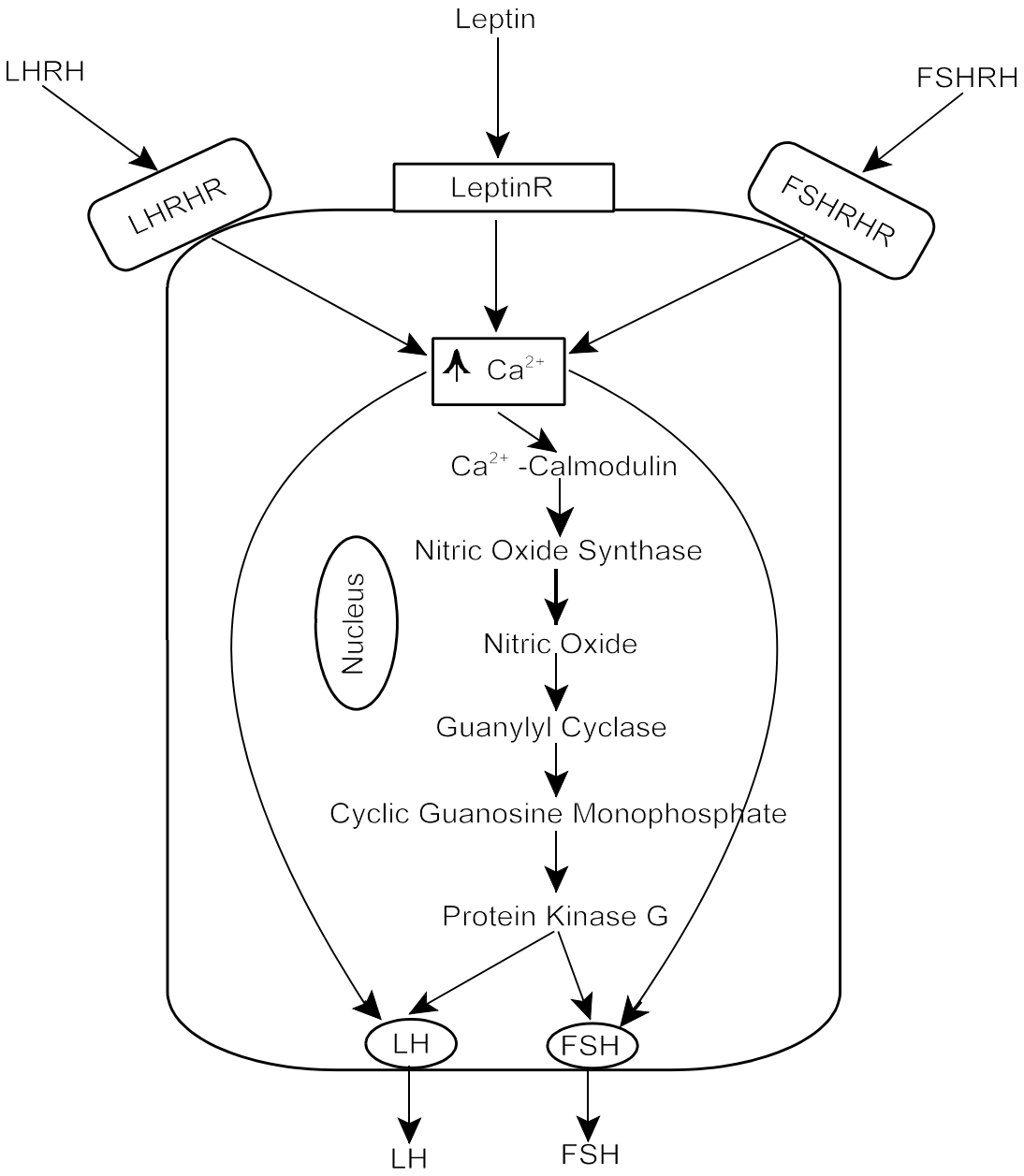


Figure 5. Diagram illustrating the mechanisms of gonadotropin-releasing action of leptin, luteinizing hormone-releasing hormone (LHRH) and follicle-stimulating hormone releasing hormone (FSHRH) at the level of the anterior pituitary. Calcium ion release may also have an independent action.

neurotransmitter in the central nervous system (Gore 2004). However, each input likely represents unique mechanisms of GnRH modulation. Glutamate, a primary excitatory neurotransmitter of the hypothalamus, has been recognized for years as having major regulatory input to GnRH neurons via glutamate receptors (Figure 6), both NMDA and non-NMDA (Ottem et al. 2002, Gore 2004). Glutamate stimulates hypothalamic release of NO synthase from the NOergic neurons, stimulating LHRH release through the release of the guanylate cyclase pathway shown in Figure 6 (Bhat et al. 1998, Dhandapani and Brann 2000). Glutamate also stimulates the release of norepinephrine (NE) from the noradrenergic neurons, which in turn acts on the NOergic neurons, also causing stimulation of guanylate cyclase (Canteros et al. 1996). Nitric oxide is also thought to release prostaglandin E2 (PGE2), which activates adenylate cyclase and ultimately protein kinase A (PKA), causing the exocytosis of LHRH (Dhandapani and Brann 2000). Opioid and γ-aminobutyric acid receptors (GABA) are associated with the inhibitory regulation of LHRH release (Figure 6), with opioid neurons being the primary component of inhibition (Bhat et al. 1998).

Testosterone is the primary hormone secreted by the adult testes (<5% from adrenals) at about 7 mg/day for young human males, decreasing in concentration with age (Goodman 2003). Cholesterol serves as the backbone of the steroid molecule, and transport of cholesterol to the inner mitochondrial membrane is the rate-limiting step of steroid hormone production for both males and females (Mathieu et al. 2002, Goodman 2003, Houk et al. 2004). Stored cholesterol may be from either *de novo* synthesis within Leydig cells, or from circulating cholesterol entering the cell by receptor-mediated endocytosis. The availability of cholesterol and the cellular composition of modifying enzymes dictates the type and amount of steroids synthesized. Movement of cholesterol across the outer mitochondrial membrane poses no problem. However, the aqueous space between the inner and outer mitochondrial membrane blocks the passage of cholesterol into the mitochondria. It is believed that the inter-membrane associated steroidogenic acute regulatory protein (StAR) transports

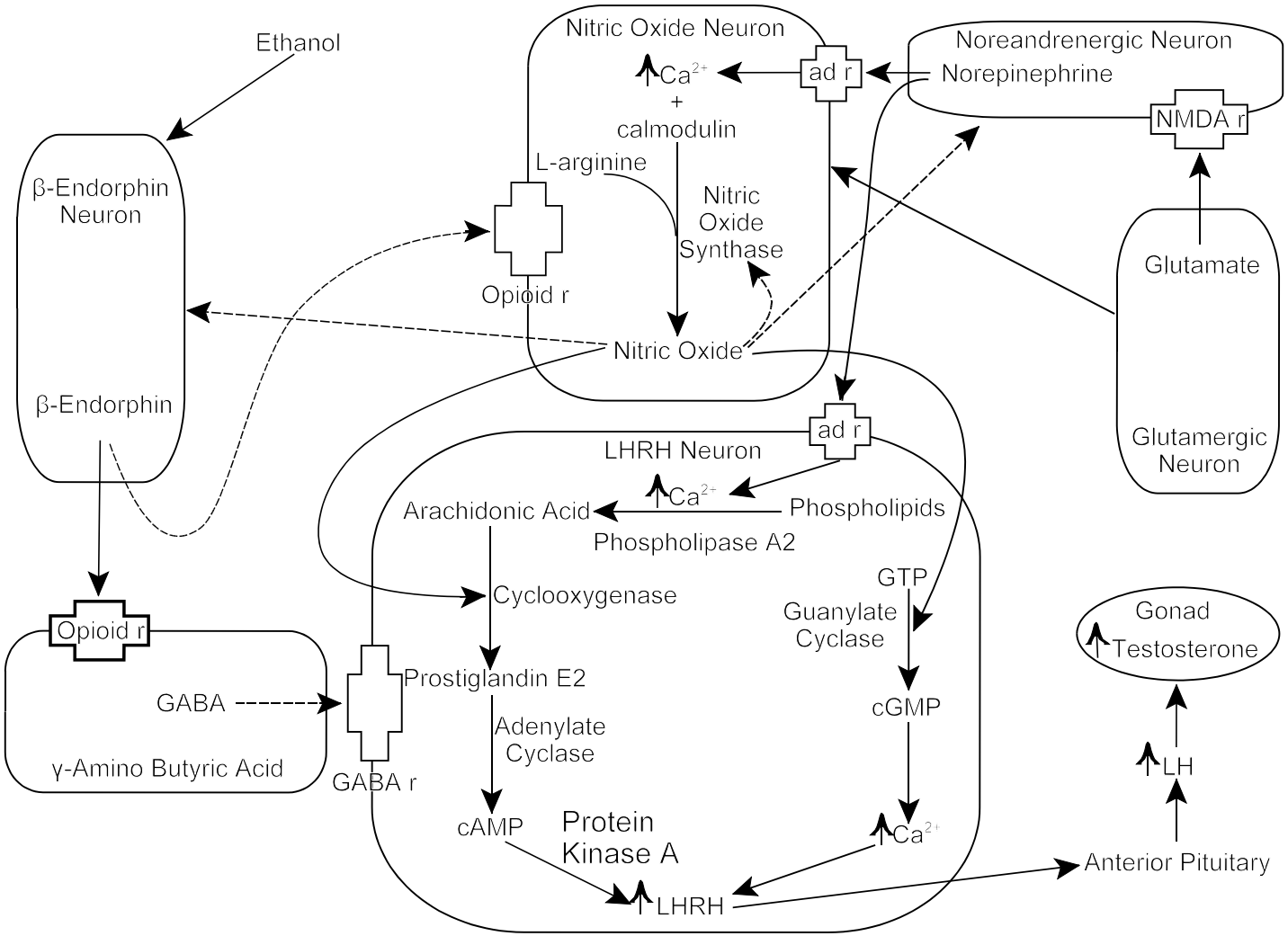


Figure 6. Diagram of excitatory and inhibitory pathways of nitric oxide synthesis and luteinizing hormone-releasing hormone release control. Solid lines are excitatory and dashed lines are inhibitory.

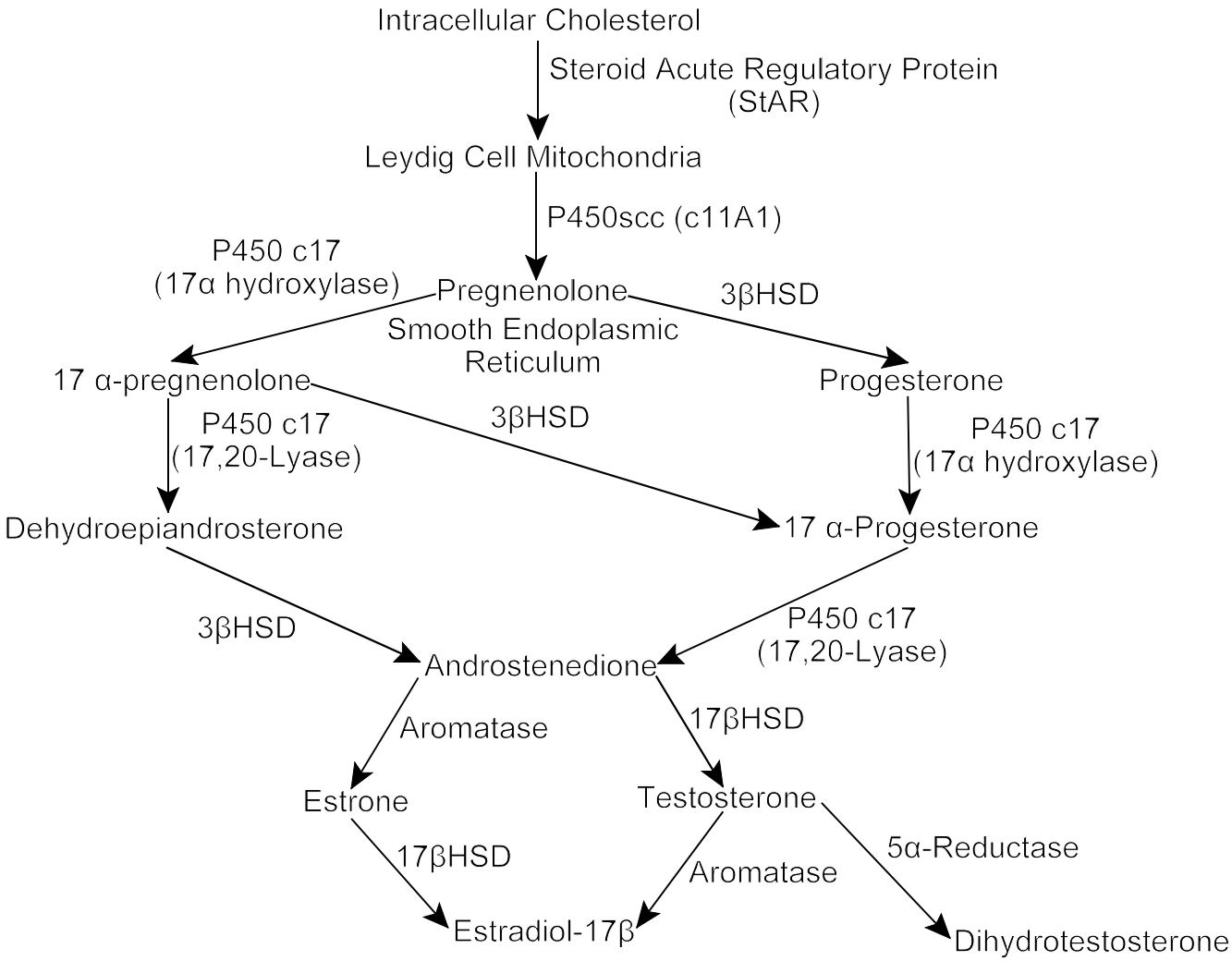


Figure 7. Biosynthesis of testicular steroids and corresponding enzymes. Testicular hormone production is almost 100% testosterone.

cholesterol to the inner membrane by an as yet undefined mechanism (Mathieu et al. 2002). Luteinizing hormone binds to its respective Leydig cell receptors, stimulating the cAMP/PKA pathway and the subsequent phosphorylation of the cyclic AMP response element binding protein which translocates to the nucleus for transcription and production of the StAR protein (Goodman 2003, Houk et al. 2004). The same pathway is also responsible for promoting steroidogenesis through gene transcription of enzymes like cytochrome P450c17 (CYP17) as well as proteins responsible for other cellular functions. Once cholesterol has been transported to the inner mitochondrial membrane for males, it is converted to T via the biochemical pathways shown in Figure 7. It appears that neither LH nor cAMP stimulate the five enzymes responsible for the conversion of cholesterol to T as the rate of synthesis is dictated by the availability of cholesterol (Goodman 2003). However, LH does sustain functional integrity of Leydig cells by maintaining appropriate enzyme levels responsible for steroid synthesis. Transcription of the gene encoding cytochrome P450c17 though, does appear to be sensitive to cAMP. Autocrine feedback inhibition of testicular steroid synthesis appears to be by T inhibiting expression of the StAR protein, thus reducing cholesterol availability (Houk et al. 2004). The negative feedback loop of steroid synthesis may also modulated with MIH which seems to inhibit cytochrome P450c17 hydroxylase/c17-20 lyase expression while enhancing cAMP-induced expression of StAR mRNA, increasing availability of cholesterol for conversion to pregnenolone (Houk et al. 2004).

Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been reported to perturb the regulation of testes steroidogenesis, possibly through the AhR (Moore et al. 1991, Fukuzawa et al. 2004, Lai et al. 2005). Moore et al. (1991) concluded that TCDD inhibited the mobilization of cholesterol to cytochrome $P450_{scc}$. However, Fukuzawa et al. (2004) reported that adult male wild-type mice exposed to TCDD showed significant testicular reduction of $P450_{scc}$ and LH receptor (LHR), while AhR null mice showed no effects from the exposure. Lai et al. (2005) later indicated that TCDD can modulate cAMP

signaling in rat Leydig cells, affecting steroidogenesis. PCBs have also been shown to diminish LHR density and reduce steroidogenic enzymes $P450_{scc}$, 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) (Murugesan et al. 2005).

The testes also produce small quantities of 17β-estradiol (E2) from T using the enzyme aromatase (P450arom or CYP19), with some androstenedione conversion to estrogen (E2) which is the extra-testicular precursor for E2 synthesis (Goodman 2003). Aromatase activity is mainly localized in the Leydig cells, with immature Sertoli cells having some capacity to convert T to E2 (Levallet et al. 1998, Carreau et al. 2003, Goodman 2003). Developing germ cells also have aromatase activity in which enzyme transcripts decrease as the cells mature to become spermatozoa, while aromatase activity increases during maturation (Carreau 2001). Estrogen receptors are found in Leydig and Sertoli cells, different developing stages of germ cells and in the efferent ductules and epididymis (Carreau et al. 2001, O'Donnell et al. 2001, Lambard et al. 2004, Lambard et al. 2005). Estrogen appears to promote spermatozoa survival, motility, capacitation and acrosome reaction (Carreau 2001, Aquila et al. 2002, Aquila et al. 2003, Carreau et al. 2003). Estrogen is also thought to regulate semen fluid volume, concentrating sperm numbers in the efferent ductules prior to entry into the epididymis (Hess et al. 2001, O'Donnell et al. 2001). TCDD and non-ortho substituted PCBs have been shown to inhibit P450arom activity (Drenth et al. 1998)

Mammalian Male Reproductive Development

Male sex differentiation begins at fertilization in mammals with the sex chromosome formation of heterogametic XY males (Hunter 1995, Kelce and Gray 1999). The combined genetic information is the basis of gonadal sex development where the SRY gene (the primary testis determinant) on the Y chromosome directs the indifferent gonads into testes. The gonads develop as stratifications (ridges) of the coelomic epithelium on the medial side of the

urogenital ridge at about the 4th week of human gestation (George and Wilson 1994). Most of the gonadal cell types are derived from the mesodermic region of the urogenital ridges. The primordial germ cells, however, arise outside of the presumptive gonad from primitive ectodermal cells of the yolk sac entoderm (epiblast). Primordial cells are identified from other embryonic cells as being large, with rounded nuclei, clear cytoplasm and containing large concentrations of alkaline phosphatase and glycogen. At the beginning of the 4th week of gestation in humans (~7th day in mice), the germ cells begin to migrate to the coelomic epithelium region of the gonadal ridges. During migration, the number of primitive cells increase by mitotic division from a few in number to thousands. The maximum number of primordial cells is ~26,000 by day 14 in mice, while the number of cells by week 6 of gestation for humans is ~3,000 and increases to ~30,00 by week 9 of pregnancy (Hunter 1995, Bendsen et al. 2003). Once the germ cell gonocytes have reach the gonadal ridges, they move from the epithelial cells to the parenchyma while taking some of the epithelial cells with them to the underlying mesenchyme. The indifferent gonad is formed by the end of the 5th week of human embryogenesis (George and Wilson 1994). The gonad is composed of germ cells, support cells of the coelomic epithelium (which give rise to either the Sertoli cells of the testes or granulosa cells of the ovary) and stromal or interstitial cells from the mesenchyme of the gonadal ridge (Leydig cells). The urogenital tract looks identical for both sexes, with the future accessory reproductive organs developing from a dual wolffian and müllerian duct system arising from mesonepheric kidney early in gestation (George and Wilson 1994). The wolffian duct system develops first from the mesonephros by the 6th week of gestation, with the subsequent development of the müllerian duct system forming from the coelomic epithelium as an evagination just lateral to the wolffian ducts. The caudal end of the müllerian duct is intimately associated with the wolffian duct during evagination, but later separates to become a separate duct system. By the end of the indifferent stage of sexual differentiation (before gestation week 8), the ducts begin to develop into the internal accessory organs

of reproduction (George and Wilson 1994). At the same time, the anlagen of the external genitalia of both sexes are also indistinguishable. The genital eminence is located between the umbilicus and the embryonic tail. The eminence consists of the genital tubercle sided by 2 prominent genital swellings, with the urogenital sinus situated between the swellings and surrounded by genital folds.

Sexual dimorphism of the human male gonads begins during the 6th week of gestation (12.5 days in the mouse) with the development and aggregation of Sertoli cells into spermatogenic cords of the fetal testes (George and Wilson 1994, Hunter 1995). Germ cells drawn to the area of spermatic cord formation join with the presumptive Sertoli cells and become situated within the developing cords. The spermatogenic cords become the future seminiferous tubules once the lumen develops and the basement membrane is formed. Normal development of the cord is also dependent on peritubular myoid cells which also arise and migrate from the mesonephric region. Testis growth at this time is largely the function of somatic and germ cell proliferation of cells already in residence of the embryonic testes and not further in-growths of mesonephric cells (Hunter 1995).

Müllerian inhibiting hormone is synthesized by the Sertoli cells shortly after the formation of the spermatic cords in the fetal testes, and acts locally to suppress müllerian duct development. The müllerian duct system is sensitive to the MIH for only a short time (<week in humans, a few days in mice and rats), and secretion of the inhibiting hormone is independent of spermatogenic tubule formation. Müllerian duct regression (7-10 weeks in humans, 13-17 days in rodents) is an active process, with duct degeneration beginning adjacent to the testes (Bédécarrats et al. 2003). Müllerian duct degeneration (apoptosis) by the hormone leads towards the differentiation of the male testes, but the inhibiting hormone may also further influence and promote the histologic and endocrine development of the testes (George and Wilson 1994). The müllerian duct almost completely disappears.

Testosterone synthesis begins during the 8th week of human gestation, shortly after differentiation of the spermatic cords and coinciding with the histologic differentiation of the Leydig cells (day 13.5 in mice) after their migration from the mesonephric region (Gondos 1980, Hunter 1995). During the onset of mullerian duct degeneration, the wolffian duct system develops into the male ejaculatory system under the influence of T, which is mediated by epidermal growth factor (George and Wilson 1994, Gupta 1996). Testosterone from the differentiated Leydig cells influences development of the wolffian duct system to become the epididymis, vas deferens, and seminal vesicles (Kelce and Gray 1999). The embryonic testes also produce the more potent androgen DHT. Testosterone is metabolized to DHT via enzymatic activity of 5∝-reductase, and both androgens bind to the androgen receptor which induces the development of the male reproductive system. It is believed that the concentration of T necessary to virilize the wolffian duct is sufficient due to the proximity of the embryonic testes. However, the external genitalia and prostate may not receive enough T to adequately influence differentiation of the external genitalia and prostate, requiring the production of DHT which has a higher affinity (10X) and thus is a more potent androgen agonist at lower concentrations than T (Imperato-McGinley et al. 1992), facilitating differentiation of the external genitalia and prostate. The ventral prostate of the rat is unique in that it also expresses high concentrations of 17β-hydroxysteroid oxidoreductase, converting T into the inactive androgen androstenedione. The rat prostate, however, also has high 5∝-reductase activity which may shunt testosterone to DHT and maintain adequate concentrations of non-aromatizable active androgen (George 1997).

The external genitalia begins to develop just after the onset of virilization of the wolffian ducts and urogenital sinus (George and Wilson 1994, Hunter 1995). The genital tubercle begins to elongate, while the urethral folds begin to fuse over the urethral groove, forming the penile urethra. The 2 genital swellings are brought together to form the scrotum. Development of the early male

genitalia occurs by the end of week 12 in humans. There is little difference in size of the genital tubercle between the 2 sexes even after the male urethra is complete. However, the male phallus grows (under the influence of androgens) in size throughout the latter phases fetal development until at birth, it is noticeably larger than the urogenital tubercle of the female. Descent of the testes into the scrotum occurs late in gestation and is initiated by insulin-like factor 3 (INSL3) (Nef and Parada 1999, Zimmermann et al. 1999). First, there is the androgen dependent degeneration of the cranial suspensory ligament (CSL) which attaches the cranial end of the testes to the abdominal wall (George and Wilson 1994, Hunter 1995, Zimmermann et al. 1999). The INSL3 dependent shortening of the caudal gonadal ligament (gubernaculum) occurs next, along with the rapid growth of the abdominal-pelvic region of the male fetus. This brings the testes to rest against the anterior wall of the abdomen in the region where the inguinal canals will form. Next, the formation of the processus vaginalis begins in which the inguinal canals and scrotum develop, with the forming of a double-layered sleeve that lines both inguinal canals and the scrotum. Increases in intra-abdominal pressure are thought to initiate herniation of the abdominal wall along the path of the inguinal portion of the gubernaculum (a embryonic structure that tows and guides the testes into the scrotum). Continued pressure enlarges the processus vaginalis around each gubernaculum, leading to the formation of the inguinal canal. The gubernaculum continues to increase in size until the diameter of the canal approaches that of the testis. Finally, the descent of the testes occurs where the abdominal testes move through their respective inguinal canals and terminate in the scrotum. This overall process is in part, regulated by androgens, especially during the formation of the gubernaculum (George and Wilson 1994, Hunter 1995). For most eutherian mammals, testes containing normal sperm-producing seminiferous tissue can only occur if they descend into the scrotum, resulting in exposure to temperatures cooler than that of the abdomen (e.g. 2-5°C cooler) (Hunter 1995).

The development of the brain is also important as it is also a sex organ in which hormone and sperm production is controlled. The pituitary consists of two lobes, of which the anterior portion forms prenatally via evagination of Rathke's pouch, while the posterior lobe arises from the ventral projection of the diencephalon (Cummings and Kavlock 2004). The differentiation of the pituitary structures are mediated by autocrine and paracrine signals from adjacent ectoderm and neural tissue (Siler-Khodr 1998), with rudimentary structures visible by weeks 4-5 of gestation in humans (day 9 in mice). The hypothalamic-pituitary complex is also forming, undergoing differentiation towards endocrine function. Production of both LH and FSH in the anterior pituitary begins around the fifth week of human gestation, with gonadotropin releasing hormone production occurring in the hypothalamus about the same time. However, the action of the releasing hormones can occur only after the formation of the HPS (Siler-Khodr 1998). As gonadal hormone production begins, interactions between the male gonads and the hypothalamic-pituitary axis occurs having reciprocal effects on development and differentiation (Cummings and Kavlock 2004). Though leydig cell differentiation occurs by the 7th to 8th week of gestation in humans (day 13 for mice), androgen production is initially under the control of chorionic gonadotropin (Marty et al. 2003). The earliest peak of T occurs after the 12th week of gestation in humans. Neonatal exposure of the hypothalamus by androgen is required for sexual differentiation of cells responsible for the production and release of LH (Cummings and Kavlock 2004).

Testosterone secretion from the fetal testes during the first half of gestation has sex-specific organizational effects on the neural circuitry in the male brain, directing sexual and behavioral differentiation (Hadley 1996, Mann and Fraser 1996). Testosterone exposure of brain regions sensitive to the androgen occurs at critical periods of differentiation, without which the male brain would fail to masculinize (a permanent effect). However, it is E2 rather than T that masculinizes the brain. The enzyme cytochrome P450 aromatase (CYP19) is found in abundance in the sexually dimorphic nucleus of the hypothalamic

preoptic area of the brain (Roselli and Klosterman 1998, Morris et al. 2004). Testosterone is able to cross the brain-blood barrier and is then aromatized to E2. Estrogen then interacts with E receptors to induce masculinization of the male fetal brain.

Spermatogenesis does not occur until puberty. The prenatal, early postnatal and prepubescent testes in humans and other mammals plays a critical role in hormone production (Marty et al. 2003). Sertoli cells are the most abundant cell type in the early postnatal testes, with limited numbers of germ cells present in a fairly undifferentiated state. Sertoli cell numbers in humans are currently thought to increase in number from late gestation to about three months of age when adult Sertoli cell numbers are achieved (Lemasters et al. 2000). Sertoli cell proliferation in the rat and mouse, however, begins around gestation day 16 and ceases around postnatal day 16 (Marty et al. 2003). Sertoli cells in humans secrete inhibin B until the ages of two to four, with MIH secretion throughout the prepubescent period. Three surges of T occurs in humans, at about the 12th week of gestation, at about three months of age post natal and at 12-14 years of age. Leydig cell proliferation in humans after birth is a continuous process beginning around the age of 2 until adulthood (rats between 14-28 days of age, mice between 21-33 days). Germ cell numbers in human male infants peak between 50-150 days of age and then decline (Mendis-Handagama and Ariyaratne 2001, Müller and Skakkebaek 1984). Spermatogonia increase 6-fold in number from birth to 10 years of age (Müller and Skakkebaek 1992), with an exponential increase at puberty along with an increase in testes size (Marty et al. 2003).

Though T plays a major role in the development of the male reproductive system early in fetal development, its expression by Leydig cells becomes quiescent shortly after birth and remains so until the initiation of puberty. Secretion of GnRH, which controls T secretion, is also suppressed until puberty as well (Kaiser and Kuohung 2005). All the elements necessary to control T production and spermatogenesis are present at birth. However, maturation of

the male reproductive system is, for the most part, arrested until the onset of puberty. It isn't until puberty that full activation of the hypothalamus-pituitary-gonadal axis occurs, resulting from the cascade of select biological events that determine if the organism is ready to be an adult (Navarro et al. 2004). What initiates the onset of puberty has been an intriguing mystery. The mechanism(s) that stimulate the resurgence of GnRH which triggers puberty's onset is poorly understood (Shahab et al. 2005). The critical position of hypothalamic GnRH in the system of chemical signals controlling the gonadotropins of the pituitary makes it a target of multiple regulators, and a wide array of exicitatory and inhibitory pathways governing GnRH secretion have been discovered over the last several years (Castellano et al. 2005). Initiation of puberty has long been associated with adequate health status and body fat reserves. An adequate circulating concentration of the adipocyte protein leptin (mentioned above) is also needed for the onset of puberty to occur, but leptin is thought not to be the trigger (Goodman 2003). Recently, a loss of function resulting from gene mutations encoding a g protein-coupled receptor (GPR54) was shown unexpectedly to be associated with the lack of puberty and hypogonadotropic hypogonadism in both humans and rodents (Navarro et al. 2004, Kaiser and Kuohung 2005, Shahab et al. 2005). With ligands for the GPR54 receptor yet to be fully defined, it was found that kisspeptins encoded by the metastasis suppressor gene (KiSS-1) had potent agonistic properties for this receptor. This receptor has been found in both the hypothalamus and pituitary, and the KiSS-1 peptide was found to potently stimulate LH and FSH secretion (Navarro et al. 2004, Navarro et al. 2005, Seminara and Kaiser 2005). It is currently thought that the hypothalamic KiSS-1/GPR54 system is a pivotal factor for the activation of GnRH neurons and the onset of puberty and adulthood (Navarro et al. 2004, Kaiser and Huohung 2005, Shahab et al. 2005).

River Otter Biology

The North American river otter belongs to the order Carnivora, family

Mustelidae and subfamily Lutrinae (Larivière and Walton 1998). The river otter is a top predator of most aquatic food chains that has adapted to a wide variety of aquatic habitats, from marine environments to high mountain lakes of North America (Toweill and Tabor 1982, Melquist and Dronkert 1987, Melquist et al. 2003). River otters exhibit differing degrees of sociality and spacing based on habitat, shelter availability, and food abundance (Reid et al. 1994). Otter home ranges are largely defined by local topography and overlap extensively within and among sexes, exhibiting varying degrees of mutual avoidance and tolerance depending on seasonal dispersion and availability of food and shelter (Reid et al. 1994). River otters may maintain territories within home ranges (based on population density, food availability, etc.) that are delineated by scent marking and latrine sites (Melquist and Dronkert 1987). Areas within territories may be used almost exclusively by the defending otter, excluding otters of the same sex (i.e., a female otter excludes other females and family groups while males exclude other males). River otters are generally considered piscivorus, eating a wide variety of fish species. But otters also eat a variety of aquatic invertebrates (crabs, crayfish and mussels), amphibians, birds, mammals and insects (Toweill and Tabor 1982, Melquist et al. 2003). This makes river otters good integrators of their aquatic environments and a useful surrogate species for determining both wildlife and human chemical exposure and potential harmful effects.

Food habits of river otter have been studied in a number of North American ecosystems. Otter prey are selected according to relative availability, with availability being a function of both local abundance and ease of detection and capture (Toweill and Tabor 1982, Melquist and Dronkert 1987). Tabor et al. (1980) conducted wildlife studies along the Columbia River from Vancouver to Priest Rapids Dam and from the mouth of the Okanogan River to Grand Coulee Dam. They noted that major summer foods of the otter consisted of carp (*Cyprinus carpio*), crayfish (*Pacifastacus leniusculus*) and (*P. trowbridgii*), suckers and centrarchid fishes. Waterfowl were considered important in the John Day Pool only, with sculpins and American shad (*Alosa sapidissima*) of

minor importance. Other prey eaten infrequently included the northern pikeminnow (*Ptychocheilus oregonensis*), salmon, birds, mammals, insects and mollusks, and judged to be a minor component of the otter diet (Tabor et al. 1980). Carp and crayfish were the most frequently eaten otter foods during the summer months on the Columbia River, but no quantification of percentage contribution was provided. Very little otter summer or winter food habit information for the Columbia River Basin and its tributaries is available to help estimate biomagnification factors from the otter's diet. Toweill (1974) presents the only other food habits data for river otter trapped in western Oregon from late November to early February. Contents of 75 digestive tracts showed that fish were the main staple of the diet, occurring in 80% of all tracts examined. Major fish families included Cottidae (31%), Salmonidae (24%), and Cyprinidae (24%). Crustaceans, amphibians, and birds were other important food items occurring in 33, 12, and 8%, respectively. Toweill (1974) reported otters trapped along the lower portion of the Santiam river (a tributary of the Willamette) during the fall/winter had consumed northern pikeminnows which were particularly abundant in the area. Studies by Greer (1955) and Melquist and Hornocker (1983) within the Columbia River Basin reported the percent incidence of the otter's fall/winter diet of largescale sucker (*Catostomus macrocheilus*), northern pikeminnow, and mountain whitefish (*Prosopium williamsoni*) to range between 19.8-53.4, 2.0-4.0, and 10.1-32.2 percent of scats collected, respectively. Fish collected along the Willamette River in 1993 for an osprey study (Henny et al. 2003) provides fish contaminant data for a major portion of the river, excluding the lower portion of the river below Newberg, OR. When considering the relative biomass of the three key fish species consumed by the osprey, the same species can contribute substantially to the otter's diet and therefore contaminant accumulation.

River otters are polygynous, with the breeding season for the more temperate regions (Oregon and Washington) occurring from late winter to late spring after parturition (Liers 1951, Hamilton and Eadie 1964, Melquist and

Hornocker 1983, Melquist and Dronkert 1987). Adult male otter gonads are reported to begin hypertrophy as early as October, remaining enlarged throughout the breeding season (Liers 1960). Estrus of female otters lasts from 42 to 46 days or longer (Liers 1951, Tabor 1974, Stenson 1985). Copulation normally occurs in the water and may last from 13 to 73 minutes (Liers 1951, Melquist and Dronkert 1987). As with most mustelids, female otters are probably induced ovulators (Mead and Wright 1983, Stenson 1985). Both female and male otters are thought to breed for the first time as two year olds, with females producing their first litter of kits (pups) at three years of age (Liers 1951, Wright 1963, Hamilton and Eadie 1964, Tabor and Wight 1977), though corpora lutea have been found in yearling females (Liers 1958, Docktor et al. 1987). Fertilized otter eggs divide to the blastocyst stage and are then held in stasis until approximately February when implantation occurs. Delayed implantation extends the gestation period from 290 to 380 days, with actual gestation approximately 60 to 65 days. Litter size is usually 2 to 4 kits, with kits cared for by the female only. Females and young stay together through the fall and winter until the juveniles are 7 to 12 months of age, dispersing prior to the female giving birth to a new litter (Melquist and Hornocker 1983).

River otters, like other mustelids, exhibit the reproductive strategy of delayed implantation or the temporary cessation of embryonic development (Thom et al. 2004). Delayed implantation (embryonic diapause) is a poorly understood strategy, yet suggests a possible selective advantage as delayed implantation is found in seven mammalian orders with evolution possibly occurring independently in many species (Renfree and Shaw 2000). Delayed implantation occurs when embryonic blastocyst(s) do not attach to the uterine wall, but remain floating in the uterine lumen for an extended period of time, consequently extending gestation (Sandell 1990). The diapause blastocysts remain undifferentiated and exhibit low metabolic activity, usually ceasing all cellular division. In species with seasonal delayed implantation (as with river otter), gestation is extended over a greater part of the year, which is similar for all

females within a population (Sandell 1990). The most widely accepted rationalization for diapause is the temporary uncoupling of mating and parturition. Mammals exhibiting diapause may be released from the potentially conflicting pressures of selecting mating and parturition times under optimal external conditions, having each taking place at independently favorable periods of the year (Thom et al. 2004). One hypothesis suggests that the uncoupling effect of diapause would enable young to be born earlier in the season (latitudinal?), thus giving offspring more time to develop before their first winter (Mead 1989). Another hypothesis suggests that the time of mating would move to an optimum interval where females would be able to select for more fit males (Sandell 1990).

As stated before, Delayed implantation arrests development of the embryo at the blastocyst stage. There are three stages of diapause: 1) entry into diapause and arresting of blastocyst cellular division, 2) maintenance of diapause, and 3) reactivation of cellular development and implantation after diapause. However, almost nothing is known about the mechanisms by which embryonic development is halted, maintained, and reactivated. It is known in some mustelid species that the corpora lutea is quiescent during delayed implantation, with a corresponding low plasma level of progesterone (Amstislavsky and Ternovskaya 2000). As the time for diapause release approaches, the corpora lutea begins to hypertrophy by increasing in both cell size and number, with a corresponding increase in progesterone. It is suggested that photoperiod dictates increasing size of the corpora lutea and progesterone secretion, inducing embryo implantation of the uterus (Amstislavsky and Ternovskaya 2000).

The baculum or os penis of the male river otter is a heterotopic bone of the penis that is derived from connective tissue (Baryshnikov et al. 2003). The baculum is situated dorsal of the urethra and medial to the corpus cavernosa (Baryshnikov et al. 2003). The river otter is thought to be an induced ovulator, meaning that the act of copulation induces ovulation through the required surge release of LH. It is unlikely, however, that baculum length is key to the

occurrence of ovulation (Lariviére and Ferguson 2002). It may be that baculum size and strength in some species like the otter insures penetration of the cervix to insure fertilization. Male river otter lack reproductive accessory glands found in other mammalian species, relying on the epididymis and the prostate to provide the necessary seminal volume (Figure 11). Therefore, any injury or reduction in baculum length may hinder successful mating. Lariviére and Ferguson (2003) suggested that induced ovulation evolved as a reproductive strategy beneficial to males (assuring egg fertilization during short mating interludes) and females (postcopulatory mate selection based on copulatory stimulation necessary to induce ovulation).

Research Statement

Research within this dissertation is part of earlier work reported in Henny et al. 1996 (described above), of which I played an integral part. A summer survey of river otter numbers in 1994-1995 revealed a relatively dense otter population that seemed well distributed throughout the Lower Columbia River, including the heavily polluted portion of the river within the Portland-Vancouver area. However, too few mink were present during the survey to adequately estimate their population density. The mink appeared to be locally extirpated from the Lower Columbia River, with few animals pioneering back into the area. Based on PCB concentrations in the livers of six mink collected from the Lower Columbia River in 1978-79 (range 0.55 to 2.1 µg/g, wet weight), it seems possible that PCBs may have contributed to their near extirpation over the last several decades (Henny et al. 1981). River otters collected at the same time from the Lower Columbia River had significantly higher PCB concentrations (1.7 to 23.0 µg/g, wet weight, n = 7) than mink, which infers that otters are less sensitive. However, three of four river otters collected in 1994-1995 on the Lower Columbia River at river mile 119.5 exhibited gross abnormalities in addition to having the highest PCB concentrations reported in the study, and in addition, high concentrations of several other contaminants seeming unique to

the location. Despite river otter being relatively abundant in the Lower Columbia River, adverse effects observed with the juvenile male otters (reduced baculum and gonad size, including the lack of apparent testes of a juvenile male collected at river mile 119.5 which also had the highest juvenile ΣPCB concentration) was surprising and a cause of concern. Though negative correlations between baculum and testes size with a number of OCs, PCBs, PCDDs and PCDFs was apparent, the small sample size and numerous contaminant correlations resulting from the complex mixture of chemicals was cause for concern. Based upon earlier work along the Lower Columbia River in 1994-1995, this study was designed to more fully understand river otters in the Pacific Northwest, and the possible role that contaminants may have on the male portion of the population. The expanded study area (required to obtain more animals without causing local extirpation due to over trapping) required several factors to be evaluated:

1. Test for potential morphometric size differences between river otter males collected from different geographical regions as a result of study area expansion.
2. Evaluate possible regional contaminant differences within the expanded study area.
3. Determine reproductive status of juvenile and yearling river otter males (pre-pubertal or pubertal?). Pubertal influences on reproductive organ growth and seasonal hypertrophy of juvenile males would further complicate evaluating possible endocrine disruption effects.
4. Evaluate chemistry residue data collected to determine if contaminants are related to reproductive organ hypoplasia. Also, evaluate reproductive organs in relation to when young were born.

To address these issues, several hypotheses (H_0) are proposed:

$H_0$1: Morphometric measurements are the same in male river otters collected from different geographical regions of the expanded study area.

$H_0$2: Contaminant concentrations are the same in male river otters collected from different geographic regions of the expanded study area.

$H_0$3: Juvenile and yearling male river otters show no evidence of puberty as signified by testes production of male androgens and spermatozoa.

$H_0$4: Reproductive organ size of juvenile river otter males is independent contaminant concentrations.

MATERIALS AND METHODS

Otter Collection and Necropsy

Trapper Caught Otters

Personnel from fur bearer programs of both Oregon and Washington Departments of Fish and Wildlife were contacted to obtain mailing lists of licensed trappers for 1994-95 and 1996-97 trapping seasons. Trappers were sent response forms to acknowledge whether or not they would like to participate in the study. Those trappers who wanted to participate were sent aluminum foil for wrapping skinned otter carcasses and identification tags. They were instructed to tag each otter with information pertaining to the trapper's name, date otter was trapped, and trap location (county, nearest town, legal description). Trappers were instructed to wrap otter carcasses individually in the aluminum foil prior to freezing. The frozen otter carcasses were held by trappers until the end of the trapping season when they were collected. Both male and female river otter carcasses were requested from trappers during the fall-winter trapping seasons of 1994-95, with only male otters requested in 1996-97.

Pelted carcasses were thawed and necropsied at the Veterinary Diagnostic Laboratory (VDL), College of Veterinary Medicine (CVM), Oregon State University (OSU), Corvallis, Oregon. Otters were sexed and evaluated for general body condition. Morphometric information was recorded (body mass,

total length, etc.). Total body length was taken from the tip of the nose to tip of the tail. Tail length was measured from the first movable joint of the tail to its tip. Neck circumference was measured midway between base of the skull and the base of the neck. Feet were checked for deformities. Dissections were then performed, with major organs weighed and measured, noting any abnormalities. Sections of the reproductive organs (gonadal tissue, prostate gland, uterus, etc.) and abnormal tissues were preserved in 10% buffered formalin for later histology. No other tissue was collected for histology if a carcass was frozen, as freezing destroys cellular structure. The reproductive organs survived freezing quite well, possibly do to a high lipid concentration. A liver sample from each otter was collected for later chemical analysis. Feces (scat) samples were collected (when available at the end of the large intestine near the rectum) for chemical analysis. The baculum (os penis), right femur, humerus, and scapula were removed from each otter and placed in a dermestid beetle colony at the Department of Fisheries and Wildlife, OSU, for soft tissue removal.

Live Trapped Otters

River otters were also live trapped during the fall-winter seasons of 1996-97, 1997-98, and 1998-99. Treadle actuated drop door box traps were used to live trap otters in boat houses and fish hatcheries. Reference otters were trapped at Woahink Lake, two miles south of Florence, OR, and at Wizard Falls Fish Hatchery, on the Metolius River, Camp Sherman, OR. Study otters were trapped in boat houses of the Multnomah County Sheriffs Department, located on the Willamette and Columbia Rivers, Portland, OR. Once an otter was trapped, it was transferred to a travel cage for transportation to the VDL at OSU. The travel cage was covered with a tarp to help calm the animal while in transit. Once at the VDL, a captured otter was transferred from the travel cage to a holding tube whose dimensions were described by Serfass et al. (1993). A plunger attachment one end of the holding tube was used to pin the otter at the other end of the tube to facilitate introduction of chemical restraint. The mass of

the otter was first estimated and an intramuscular injection of the dissociative ketamine hydrochloride (~22 mg/kg body mass) was used for immobilization (Serfass et al., 1993), with further analgesia and sedation using xylazine at ~0.5 mg/kg body mass (as per label). Immobilization usually occurred within five minutes of injections. The sedated animal was then removed from the holding tube and transferred to the VDL necropsy lab.

While immobilized, otters were sexed and evaluated for general body condition. Morphometric information were recorded (body mass, total length, etc.). Total body length was taken from the tip of the nose to tip of the tail. Tail length was measured from the first movable joint of the tail to its tip. Neck circumference was measured midway between base of the skull and the base of the neck. The feet were checked for any deformities.

Blood was collected from an anesthetized otter by cardiac puncture at a position directly under the sternum, using the intercostal spaces between the 7^{th} and 11^{th} ribs. A 10 ml syringe with a 3.8 cm long 18 gauge needle was used to take blood samples. Blood was transferred to two 10 ml sterile non-additive and one 10 ml EDTA Vacutainer® vials. The EDTA blood sample was gently inverted several times to thoroughly mix the blood with the additives prior to refrigeration. The non-additive vials set for two hours at room temperature, allowing adequate time for clotting. The clotted blood was then centrifuged for 15 minutes at 2,000 rpm, with the separated serum drawn off and transferred to two 5 ml cryogenic vials. One of the vials was refrigerated for blood chemistry to be completed within 48 hours after collection, while the second vial was frozen and stored at -80° C . The EDTA blood sample was refrigerated immediately and used within 48 hours for hematology. Three blood smears were prepared and allowed to dry for differential blood cell counts.

Once blood samples were taken from an anesthetized otter, it was then euthanized by exsanguination using the heart puncture needle. Once the heart stopped beating, the body cavity was opened to examine and remove major organs for weighing, noting any abnormalities. Sections of normal and abnormal

tissues were preserved in 10% buffered formalin for later histology. A liver sample from each otter was collected for later chemical analysis. The baculum (os penis), right femur, humerus, and scapula were removed from each otter and placed in a dermestid beetle colony at the Department of Fisheries and Wildlife, Oregon State University, for soft tissue removal. The pelt was removed and weighed to adjust the body mass for comparison with trapper caught skinned carcasses.

Age Determination

An upper canine tooth was used to determine the age of each river otter using microscopic analysis of the tooth's cementum annuli. Each tooth was extracted using pliers by first heating the otter skulls in almost boiling water for 15-30 minutes. The teeth were cleaned and placed in individual pre-labeled envelops prior to shipping to Matson's Laboratory LLC (Box 308, Milltown, Montana, 59851) for aging. Stephenson (1984), Fancy (1980) and Matson (1980) describe preparation of the teeth. Briefly, the root of each tooth was removed and decalcified in 5% nitric acid for 24 hours using light mechanical agitation. Tooth specimens were then cut sagittally, washed in water, and cleared with 70% ethanol. Half of each specimen was further dehydrated through increasing strengths of ethanol and cleared in xylene prior to infiltration and imbedding in paraffin using standard histological procedures. Several longitudinal sections of each tooth specimen were made at 15 μm thickness, with 3 to 5 sections of each specimen placed on a standard microscope slide for staining using a hematoxylin stain. Coverslip mounted slides were examined using a light microscope at 100x magnification.

After age determination, the otters were grouped into three age classes (juvenile, yearling and adult) for data analyses. Juveniles were young of the year at the time of collection during the fall-winter trapping season, with yearlings representing greater than one year, but less than two years of age. Adults included animals two years old and older.

Gonadal Steroid Hormone Analyses

Testosterone concentrations were determined in river otter testes using a modified method of Van Look et al. (1989) and Verbeke (1979). Standards of 4-androstene 3,17-dione (androstenedione), 17β-hydroxy-4-androsten-3-one (testosterone), 11α-hydroxy-4-pregnene-3,20-dione (hydroxyprogesterone) and 4-pregnene-3,20-dione (progesterone) were used to develop the extraction method. Bovine testis was then used to test the extraction method using androstenedione, hydroxyprogesterone and progesterone as internal standards.

Testosterone was quantified from testes samples of male otters selected throughout the trapping period from each of the three age categories described above to evaluate testosterone levels as otter males come into breeding. Gonad tissue from each otter was finely minced and homogenized using a glass mortar and pestle. An approximate 2 g sub-sample from each testes sample was placed into an individual 50 ml screw cap glass centrifuge tube to which was added 1 ml of MilliQ purified H_2O (hereafter just H_2O). The water was thoroughly mixed with the sample using a vortex shaker. To the sample was added 30 ml of HPLC grade hexane. The glass centrifuge tube was then capped and placed on a vortex shaker for 1 minute to thoroughly mix the sample with the hexane. The sample was then placed in a 37°C water bath for 5 minutes. The sample was again vortexed for 1 minute and placed in the water bath for another 5 minutes, with the process repeated once more. The sample was then removed from the water bath and centrifuged at 3000 rpm in an IEC Model CL benchtop centrifuge for 5 minutes and sample extract transferred to 250 ml round bottom rotary evaporator flask. The testes sample was then re-extracted with an additional 30 ml of hexane as described above.

After the second 30 ml volume of sample extract was transferred to the 250 ml rotary evaporator flask, the flask was placed on a rotary evaporator apparatus to remove the hexane from the sample extract, taking the sample almost to dryness at 50°C. The sample was transferred to clean 50 ml screw cap glass centrifuge tube using five 1 ml aliquots of hexane. To the sample was

added 25 ml of HPLC grade methanol (MeOH), with the sample solvents thoroughly mixed and allowed to stand for 10 minutes. To the sample was carefully decanted 12 ml of 0.04 M sodium acetate adjusted to a pH of 5.2. The two phases were allowed to separate and the hexane phase containing lipids was pipetted off. The turbid MeOH phase was partitioned three more times against 5 ml of hexane by mechanical shaking, each time discarding the hexane phase. The two phases were separated each time by centrifugation for 5 minutes at 3000 rpm.

The MeOH-sodium acetate sample extract was transferred to a 125 ml separatory funnel and 30 ml of dichloromethane (DCM) was added. The two phases were thoroughly mixed for 1 minute and the phases allowed to separate. The bottom phase of DCM containing the hormones was collected into a 250 ml round bottom rotary evaporator flask. The MeOH-sodium acetate phase was partitioned again using another 30 ml of DCM. The second DCM phase was added to the first. The sample extract was then rotary evaporated to remove the DCM to almost dryness at 50°C.

The hormone sample was transferred from the rotary flask, using five 1 ml aliquots of 11% MeOH-H_2O, to a 6 ml solid phase extraction cartridge (SPE) containing 500 mg of C18 sorbent used for non-polar reverse phase sample cleanup. The cartridge was first pre-washed with 3 ml of MeOH and then with 3 ml of H_2O prior to sample transfer. A light vacuum was applied to the cartridge containing the C18 sorbent to facilitate sample loading. While under vacuum, the cartridge containing the hormone sample was washed first with 3 ml of H_2O, then with 3 ml of 55% MeOH-H_2O, and finally with 1.5 ml of hexane. The sample was then eluted into a 50 ml round bottom rotary evaporation flask using 6 ml of MeOH and the sample evaporated to dryness. The hormone extract was re-dissolved in 500 µl of MeOH and transferred to a Waters 2 ml high performance liquid chromatography (HPLC) vial fitted with a 200 µl insert.

Hormone samples were analyzed on a Waters 1690 separations module connected to a Waters 996 photodiode array detector. Samples were loaded

onto an auto-sampler cassette and placed in the sample chamber of the separations module which was held at 5°C. Two solvent mixtures were used for the HPLC analysis. Solvent A consisted of 62% MilliQ H_2O, 28% MeOH, 5% acetonitrile (CH_3CN), and 5% iso-propanol. Solvent B consisted of 35% MillQ H_2O, 45% MeOH, and 20% butanol. The HPLC column used for the hormone analysis was a Waters 3.9 x 150 mm Nova-Pak® C18 60 Å, 4 µm column, held at 35°C. Initial conditions of the HPLC was 100% solvent A with a flow rate of 0.8 ml per minute. The column was equilibrated with 100% solvent A for five minutes prior to sample injection. A 50 µl injection of the hormone sample was made with an isocratic run started using 100% solvent A for 15 minutes. A gradient was then run for 21 minutes, increasing the percent of solvent B by 2.5% per minute until a final of 48% solvent A and 52% solvent B was reached. The run was held at the final solvent percentage for 4 minutes and then the column flushed with 100% MeOH for two minutes before returning to 100% solvent A and re-equilibration for 5 minutes before the next injection. The photo diode array was set at 246 nm to detect the hormones androstenedione, testosterone, hydroxyprogesterone and progesterone. The lower level of quantitation was at 20 ng g^{-1} wet weight (ww) for all four steroid hormones.

Contaminant Chemical Analyses

Organic Chemistry

Liver samples collected from river otters were sent to the Great Lakes Institute for Environmental Research (GLIER) at the University of Windsor, Windsor, Ontario, Canada, for contract chemical analyses as described in Lazar et al. (1992) and GLIER (1995). Samples were analyzed for 20 organochlorine pesticides and metabolites (OCs) and 43 polychlorinated biphenyls (PCBs)

congeners. Moisture content was determined by oven-drying a 1 g sub-sample of animal tissue sub-sample in a pre-weighed aluminum weighing boat for 24 hr at 125°C. For OCs and PCBs, a sample of animal tissue homogenate (~5 g) was ground with anhydrous Na_2SO_4 (5 fold the sample weight) using a glass mortar and pestle. The free-flowing powder obtained was extracted using dichloromethane (DCM)/hexane (50% V/V). The eluate collected was concentrated to approximately 5 ml after addition of 5 ml isooctane, and adjusted to 25 ml using hexane. Lipid determination was made by drying 2 ml of the sample eluate in a preweighed glass beaker at 105°C for 1 hr. The remaining 23 ml of extract was concentrated to ~2 ml after adding 5 ml of isooctane. If the lipid content of an extract was higher than 0.5 g/sample (i.e., fat samples), the extract was placed on a gel permeation column (GPC) for bulk lipid separation after addition of 2 ml DCM. A total of 300 ml 50% DCM/hexane (v/v) was added to the GPC and eluted. The first 130 ml eluate containing the lipid was discarded. The last 170 ml of eluate containing the contaminants of interest was collected and 5 ml of isooctane added. The sample was concentrated to ~2 ml and transferred to a florisil column for additional cleanup. Samples containing less than 0.5 g fat/sample were also transferred to florisil column for additional cleanup. The first fraction from the florisil column was collected using 50 ml of hexane, with subsequent second and third fractions collected using 50 ml of 15% DCM/hexane (V/V) and 50 ml of 50% DCM/hexane (V/V), respectively. The 3 fractions were each concentrated to ~2 ml after addition of 5 ml of isooctane. The fractions were then adjusted to a suitable final volume in isooctane. Final dilutions of the sample fractions contained the following:

Fraction 1	Fraction 2
1,2,4,5-tetrachlorobenzene (1,2,3,4-TCB)	α-hexachlorocyclohexane (HCH)
1,2,3,4-tetrachlorobenzene (1,2,4,5-TCB)	β-HCH
pentachlorobenzene (QCB)	γ-HCH
hexachlorobenzene (HCB)	oxychlordane
octachlorostyrene (OCS)	trans-chlordane
trans-nonachlor	cis-chlordane
p,p'-dichlorodiphenyldichloroethylene (DDE)	p,p'-dichlorodiphenyldichloroethane (DDD)
photomirex	p,p'-dichlorodiphenyltrichloroethane (DDT)
mirex	cis-nonachlor
PCBs (including mono-ortho congeners)	PCBs (non-ortho-substituted congeners)

Fraction 3
heptachlor epoxide
dieldrin
trichlorophenylmethanol

A 5% carbon/silica gel mixture was used to separate the non-ortho-substituted PCBs from florisil fraction #2. An ~2 ml concentrated extract of fraction #2 was added to the top of a previously prepared carbon/silica column. The first fraction was eluted using 30 ml of hexane, followed by elution of a second fraction using 30 ml of DCM. The column was then inverted and a third fraction eluted using 30 ml of toluene. The third fraction (containing the non-ortho-substituted PCBs) was concentrated and reconstituted to an appropriate volume using isooctane. Fractions from the florisil and carbon/silica separations were run separately on a Hewlett Packard (HP) Model 5890 gas chromatograph, equipped as follows:

^{63}Ni-electron capture detector (ECD)
HP-3396 Integrator
HP-7673A Autosampler
Column: 30 m x 0.25 mm I.D. x 0.25 μm DB-5 film thickness (J&W)
Injector temperature: 250°C; Detector temperature: 300°C
Carrier gas: helium at ~30 cm/sec - determined at 100°C (1 ml/min)
Make-up gas: argon/methane (95%/5%) at 50 ml/min

Oven temperature Program:
Initial temperature: 100°C Initial time: 1 min
Rate: 10°C/min to 150°C, then 3°C/min to 275°C
Final hold time: 5 min Equilibrium time: 3 min
2 µl sample injection using a splitless injection mode

Quantification was accomplished by comparing sample-peak area against standard-peak area of three standards supplied by the Canadian Wildlife Service. The limit of quantitation (LOQ) for OCs and PCBs was 0.01 µg kg^{-1}, ww. OC and PCB samples were confirmed using gas chromatography/mass spectrometry (GC/MS). Extraction and cleanup methods were checked by running sample blanks, replicate samples, and certified reference samples provided by the Canadian Wildlife Service Laboratories. All PCB congeners are numbered using the International Union of Pure and Applied Chemistry (IUPAC) nomenclature. The term ΣPCBs is the concentration sum of all PCB congeners analyzed.

Otter liver samples sent to GLIER were also analyzed for 4 non-ortho substituted PCBs, 7 polychlorinated dibenzo-*p*-dioxins (PCDDs), and 10 polychlorinated dibenzofurans (PCDFs) as described in GLIER (1995). Extraction of co-planar PCBs, dioxins, and furans was similar to the previously described extraction and cleanup method with a few exceptions. First, a 10 g sample of liver homogenate was used instead, with the fat sample size remaining the same. Second, all sample extracts were run through bulk lipid separation by GPC. Third, during the florisil cleanup only two fractions were collected. The first fraction was collected using 50 ml hexane (fraction discarded), while the second fraction was collected using 100 ml toluene. The second fraction containing the contaminants of interest was concentrated and solvent exchanged into DCM to a final volume of 1 ml. The concentrated fraction was taken through a carbon chromatography cleanup process using a semi-automated high pressure liquid chromatography apparatus. Of four fractions

collected, two fractions containing contaminants of interest were concentrated using DCM prior to analysis by high resolution gas chromatography/ mass spectrometry using a VG AutoSpec-Q mass spectrometer connected to a Hewlett Packard 5890 gas chromatograph. Calculated ^{13}C-non-ortho PCB and PCDD surrogate recoveries were based on the performance standard's response, with native compounds corrected for surrogate recoveries. Quantification of sample compounds was based on the relative response of the corresponding surrogate and native congeners in the calibration standard(s). The LOQ of 0.01 ng kg^{-1} (ww) was defined as three times the background noise in the region of the ^{13}C-non-ortho PCB and PCDD surrogate quantification peaks. The TCDD toxic equivalent (TEQ) for each sample was derived from toxic equivalency factors (TEFs) suggested by Van den Berg et al. (1998) for PCBs, PCDDs, and PCDFs.

Liver samples of 40 river otters (38 adult males, 2 adult females, 1 yearling male) from western Oregon and Washington were sent to the Aquatic Toxicology Laboratory at Michigan State University for butyltin analyses. Otters used for the analyses were selected from bays and small rivers (some ship and boat use), large rivers (ship and boat use), and small creeks, small rivers, and lakes (small boat to no boat use). The analytical method used for tributyltin (TBT), dibutyltin (DBT), and monobutyltin (MBT) are described in Iwata et al. (1994) and Kannan et al. (1999). Briefly, 5 g liver samples were acidified using 10 ml of 1N HCL. Each sample was homogenized and extracted with 80 ml of 0.1% tropolone-acetone. The sample extract was then transferred to 100 ml of tropolone-benzene and eluted through packed glass column containing 35 g of anhydrous Na_2SO_4 to remove moisture. The concentrated extract was propylated with 2 ml of *n*-propyl magnesium bromide as a Grignard reagent (~2 mol l^{-1} in a tetrahydrofuran (THF) solution, Tokyo Chemical Industries, Portland, OR). The derivitazed extract was cleaned up using a glass column wet packed with 6 g of florisil. The hexane eluate was rotary-evaporated to a final 5 ml volume.

Chromatographic separation and quantification of butytins in liver samples was done on a Hewlitt-Packard 5890 series II gas chromatograph equipped with a flame photometric detector (FPD), using a 30 m x 0.25 mm (i.d.) DB-1 capillary column. Initial oven temperature at the time of injection was 80°C with a 1 min hold, followed by a programmed temperature increase of 15°C min^{-1} to 160°C and then a 5°C min^{-1} temperature increase to a final of 260°C with a 5 min hold. Injector and detector temperatures were held at 200 and 270°C, respectively. The FPD was equipped with 610 nm bandpass filter and the detector flame was a hydrogen-air-nitrogen gas mixture.

One hundred nanograms each of butyltin chloride, dibutyltin dichloride and tributyltin chloride were spiked into lake trout liver (*Salvelius namaycush*) which had butyltin concentrations below the detection limit. The spiked liver tissue was run through the complete analytical procedure and used as an external standard. Freshly derivatized external standards were run along with every set of eight otter samples to estimate butyltin concentrations by comparing peak heights of the samples to that of the external standards. Tributylhexyltin was added to each liver sample as an internal standard prior to tissue extraction. A procedural blank was analyzed for every eight samples to look for interfering chemicals and to correct for sample values when necessary. Monobutyltin was found at trace levels in the reagent blanks. Monobutyltin is used as a stabilizer in the production of polyvinylchloride (PVC) plastics. Contamination of the reagent blanks may have occurred via PVC exposure to commercial solvents. Sample values were corrected for MBT blank concentrations. No reagent blanks contained traces of tributyltin. The LOQs for MBT, DBT, and TBT were 7, 2.4, and 1 ng g^{-1} ww, respectively. Average recovery rates of monobutyltin tricloride, dibutyltin dichloride, and tributyltin chloride spiked in reference trout liver was 90 - 110% for each compound. All concentrations reported refer to the butyltin species as the corresponding ion, and samples were not corrected for recovery of the internal standard (85-105%).

Liver samples from 20 river otters (19 adult males and 1 yearling male)

collected from western Oregon were sent to the Aquatic Toxicology Laboratory at Michigan State University for analysis of perfluorooctanesulfonate (PFOS) and related fluorinated hydrocarbon compounds using the method described in Hansen et al. (2001) and Kannan et al. (2002). Briefly, a 1 g liver sample was homogenized in 5 ml of MilliQ water. A 1 ml aliquot of the liver homogenate was pipetted into a 15 ml polypropylene tube to which was added 1 ml of 0.5 M tetrabutylammonium hydrogen sulfate solution (adjusted to pH 10) and 2 ml of 0.25 M sodium bicarbonate buffer. After the sample was thoroughly mixed, 5 ml of methyl *tert*-butyl ether (MTBE) was added and the sample mixture shaken for 20 min. Centrifugation was used to separate the aqueous and organic phases, with an exact 4 ml of MTBE pipetted into a second polypropylene tube. The aqueous phase was rinsed and separated twice with MTBE, combining the organic phase and rinses. The MTBE containing the sample extract was evaporated to dryness under a stream of nitrogen and reconstituted with 0.5-1 ml of MeOH. The sample was vortexed for 30 seconds and passed through a 0.2 µm nylon filter into a HPLC autosampler vial.

Samples were analyzed on an Hewlett Packard HP1100 HPLC interfaced with a Micromass (Beverly, MA) Quattro II atmospheric pressure ionization tandem mass spectrometer operated in the electrospray negative mode. An injection of 10 µl of the sample extract was loaded onto a low volume injection loop. Chromatography was performed on a 50 x 2 mm (5 µm) Keystone Betasil® C_{18} column at room temperature. The column was conditioned with a mobile phase made up of 2 mM ammonium acetate/10% MeOH at 300 µl min^{-1}. A gradient was used, increasing MeOH to 100% in 11.5 min before returning to original conditions. The limit of quantitation (LOQ) was variable, dependent on instrument conditions and samples. Sample concentrations of perflouro-compound LOQ were compared to a standard curve calibration. A sample within the standard curve was acceptable if (1) it was back calculated to be within 30% of the theoretical value when evaluated versus a 1/x weighted curve, and (2) the peak area of the standard was at least 2 times greater than the matrix blank.

Concentration/dilution factors were included for the calculation of the LOQ. The LOQ for PFOS was 7 ng g^{-1} ww, while the LOQ for perfluorooctanesulfonamide (FOSA), perfluorohexanesulfonate (PFHxS), and perfluorooctanoate (POFA) varied from 4.5 to 75 ng g^{-1} ww. Data quality assurance and quality control protocols included matrix spikes, laboratory blanks, and continuing calibration verification. Recoveries for spiked samples ranged from 85 to 101%. Reported concentrations of PFOS were not corrected for recoveries.

Metals Chemistry

Otter liver samples were also analyzed at GLIER for 17 metals by atomic absorption spectrophotometry as described in GLIER (1995). Analyses for 10 heavy metals were run on individual liver samples. For total mercury (Hg) analyses, a 1 g sample was digested in 15 ml of a 2:1 solution of sulphuric and nitric acids at 60°C. Once completely digested, 20 ml of 5% potassium permanganate was added, followed by a 20 ml addition of 5% potassium persulphate. Finally, 5 ml of 10% hydroxylamine hydrochloride-sodium chloride was added. The sample was adjusted to 100 ml using distilled water. Total mercury was determined using flameless atomic absorption spectrophotometry, with a LOQ of 0.1 µg g^{-1} dry weight (dw). Mercury was analyzed only as total mercury.

For other metal analyses, a 2 g sample was placed into a 50 ml beaker. At room temperature, 5 ml of a 1:1 mixture of concentrated sulfuric and nitric acids was added to the sample. The sample was heated to 120°C for 1 hr and the beaker uncovered and heated at 120°C for 2 hrs or until the sample was charred. Another 5 ml of concentrated nitric acid was added and the sample heated at 120°C for 4 hr. After cooling, 30% hydrogen peroxide was added and the sample heated. The nearly colorless solution was adjusted to 100 ml using distilled water. Atomic absorption spectrophotometry was used to determine metal concentrations in tissue preparations. Metals analyzed were aluminum (Al), arsenic (As), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr),

copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), nickel (Ni), potassium (K), sodium (Na), vanadium (V), and zinc (Zn), with LOQs of 10.0, 5.0, 5.0, 0.5, 1.0, 5.0, 10.0, 5.0, 10.0, 1.0, 1.0, 1.0, 50, 50, 5.0, and 1.0 μg g^{-1} (dw), respectively.

Scat Analyses

Besides fecal samples collected from otters during necropsy, scats were also collected from latrine sites along the Willamette River, representing several individuals at each site. Twenty eight scat samples collected from Oregon and Washington were analyzed for OCs and PCBs, while samples (n=9) from heavily polluted areas were also analyzed for co-planar PCBs, PCDDs and PCDFs. Scat contaminant concentrations are reported on a lipid weight (lw) basis.

Statistical Analyses

Statistical analyses were performed using Statistical Analysis Systems (SAS), 2001. River otter morphometric data were compared between Oregon and Washington to look for geographic and latitudinal differences based on age (juvenile, yearling, and adult) for male river otters. The morphometric data were not logged and the General Linear Model Procedure (GLM) was used for both a 1-way and 2-way Analysis of Variance (ANOVA) as the data were unbalanced. Possible differences between both age and state were examined. If no significant differences were found between Oregon and Washington for each age category, data were combined. Regressions (using date of capture) were performed for significant morphometric differences to see if the differences were related to seasonal changes in morphology. Liver and kidney somatic indexes were also calculated by dividing the body mass (g) into the liver and kidney masses (g) and multiplying by 100 to obtain the index.

Residue concentrations were $\log_{10}$ transformed and presented as geometric means. The LOQ was halved and used for samples in which a contaminant residue was not detected. This value was then used for non-

detects to calculate geometric means when $\geq$50% of the samples contained quantitated residues. Because of unequal sample sizes, the General Linear Models Procedure was used for analysis of variance. Tukey's Studentized Range Test (P = 0.05) was used to separate means. Unless otherwise noted, statistical significance was set at P = 0.05.

The TCDD toxic equivalents (TEQ) were calculated for each liver sample using toxic equivalency factors (TEFs) suggested by Van den Berg et al. (1998) for selected PCBs, PCDDs and PCDFs. TEQs were originally developed for application in risk assessment by converting residue data of complex mixtures into TEQs based on their relative toxicity with respect to TCDD, which is generally recognized as the most toxic halogenated aryl hydrocarbon. This approach involves the receptor mediated mechanism of action of the Phase I metabolizing enzymes (e.g., cytochrome P450 (CYP) 1A1 or CYP1A1) involving the Ah receptor. TEQs were derived separately for PCBs, PCDDs, and PCDFs, and total TEQs determined by summation of the three contaminant categories. The TEQ values are presented on a ng kg^{-1} (ww) basis and $\log_{10}$ transformed prior to statistical evaluation.

Correlation matrices were constructed using the $\log_{10}$ (dw for metals and ww for others) residue concentrations for metals, OCs and their metabolites, PCBs, PCDDs, and PCDFs with adult male river otters only. The analysis was conducted to determine if specific contaminants could be associated with any effects shown, especially potential effects on male otter reproductive system. As was shown in Henny et al. (1996), many of the contaminants are highly correlated which makes it difficult to attribute response to one contaminant.

Seven regional locations of western Oregon and Washington were delineated based on whether or not these locations were associated with major population centers, industry or agriculture (Figure 8). The locations were the Oregon coast range (OC), the Willamette River Basin (WB), the Lower Columbia River below Bonneville Dam (LCR), southwestern Washington (SW), Olympic Peninsula , WA (OP), Puget Sound, WA (PS), and western Washington east of

the Puget Sound (PS). The General Linear Model Procedure (GLM) was used for the Analysis of Variance (ANOVA) to test for contaminant concentration differences between locations for adult and juvenile male river otters.

Regression analysis was used to evaluate contaminant concentrations in relation to morphometric measurements for both juvenile and adult male otters. A multiple regression using male otter total body length to adjust for organ-body size relationships. A multiple regression model with regional location as a random effect (i.e., accounting for measurements of otters from the same region which might be correlated) was also used to evaluate contaminant effects on the dependent parameters of morphometric measurements, especially for testis mass, baculum length, baculum mass, and prostate mass of juvenile male otters collected from both Oregon and Washington. The regression model used the seven regional locations delineated above. The model looks at the individual parameters and their interactions to test for contaminant effects on the dependent parameters. The model derives and estimates and lower and upper 95% confidence limits of the estimated change in the reproductive parameter associated with a 100-fold increase in contaminant concentration after adjusting for the body length index. The P-value of the model tests that the change is actually zero, with alpha set at 0.1. A multiple regression model was also used to see if adult male baculum size was affected by those contaminants analyzed, using body length of adult males to adjust for size differences. Other reproductive tissues were not tested as they were influenced by seasonal reproductive organ hypertrophy.

RESULTS

A total of 303 river otters (254 males and 49 females) were collected from Oregon and Washington during the fall/winter trapping seasons of 1994-95, 1996-97, 1997-98, and 1998-99, and included 76 juveniles, 54 yearlings, and 173 adults (Figure 8). Of those, nine were live-trapped, including 5 male and 4 female otters. Locations of live-trapped otters are shown in Table 1.

Table 1. Identification code, year trapped, age, sex and trap location of live-trapped otters.

Code	Trap Year	Age (years)	Sex	Trap Location
RAG-025	96/97	1	♂	Fremont Bridge, Portland, OR
RAG-031	96/97	3	♀	Woahink Lake, Florence, OR
RAG-085	96/97	1	♀	Newberg, OR
RAG98-01	97/98	0	♂	Fremont Bridge, Portland, OR
RAG98-02	97/98	5	♂	Wizard Falls Hatchery, Sisters, OR
RAG99-01	98/99	1	♂	Woahink Lake, Florence, OR
RAG99-02	98/99	1	♀	Chinook Landing, Troutdale, OR
RAG99-03	98/99	1	♂	Fremont Bridge, Portland, OR
RAG99-04	98/99	1	♀	Fremont Bridge, Portland, OR

Pathology Findings

There was moderate to extensive autolysis in all river otters, except those live-trapped. The majority of otters were in good body condition at the time of necropsy, having light to heavy fat deposits. Two yearling male otters (OT-04 at river mile 53.9 of the LCR, RAG-074 near Gaston, OR) were the only otters to have very little body fat, though no obvious injury or infection was observed. River otters with light to moderate fat deposits, had subcutaneous fat located primarily at the base of the tail and caudally on the rear legs, with fat deposition extending anteriorly over the back and abdominal muscles for otters considered to have greater than moderate fat deposits. During necropsy, however, several gross pathological findings were noted. The bacula of 25 (9.9%) male otters (8 juveniles, 5 yearlings, 12 adults) were previously fractured, which had healed or were in the process of healing as was the case with RAG98-04 (Figure 9), a juvenile otter trapped from Sauvie Island on the Lower Columbia River. Fractures occurred along the length of the bacula, but were not isolated to a particular region (i.e., proximal versus distal). A chi-square test found that the incidence of baculum fractures was independent of age, with fractures occurring

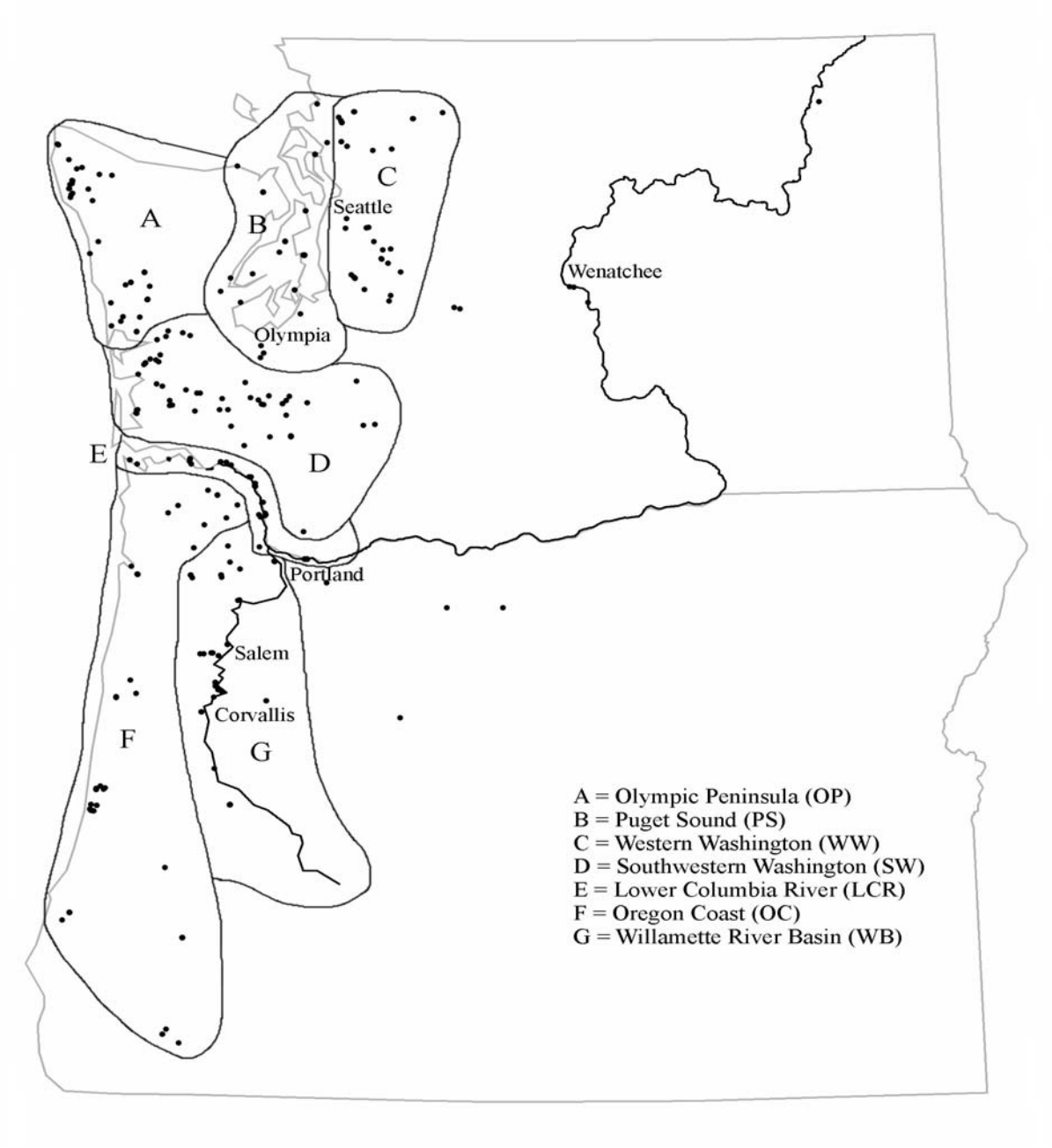


Figure 8. Map of the study area. The seven regional areas used in this study are outlined and labeled. Dots represent locations where otters were trapped.

Figure 9. Broken bacula of river otter males collected from Oregon and Washington, 1994-99.

Figure 10. Broken right humerus of river otter RAG-170 from northwestern Oregon.

in juvenile and possibly yearling age classes (P = 0.71) and not as adults. There was no significant difference in geometric mean concentrations of DDE, dieldrin, and SPCBs, and OCDD between juvenile males with (36.2, 2.3, 110 μg kg^{-1} and 74.7 ng kg^{-1}, respectively, n = 8) and without (20.1, 2.0, 86.5 μg kg^{-1} and 30.9 ng kg^{-1}, respectively, n = 55) broken bacula (P = 0.285, 0.731, 0.597, and 0.2115, respectively). However, the contaminant concentration at the time the animal was collected was not related to when the baculum was broken.

A four year old male (RAG-170) was found to have a complete closed fracture of the right humerus which was partially fused together (Figure 10). The diaphysis and proximal metaphysis of the right humerus was markedly distorted

by excessive bony callus. The site was believed to demarcate the non-union of closed fracture, as there was obvious malalignment of the fracture ends. A two year old female (OT-37) collected on the Columbia River at river mile 119.5 (Troutdale, OR) had a multilocular cystic abscess in the perineal region which measured 8.9 cm long by 5 cm deep. A three year old male (OT-38) collected from the same location exhibited left adrenal and renal agenesis. The remaining right kidney and adrenal gland was twice the normal size, exhibiting compensatory hypertrophy. Also from the same location, a juvenile male otter (OT-36) was found with no visible external or internal testes. Just down stream, a juvenile female (RAG99-02) live-trapped at Chinook Landing boat launch had the end of its tail missing and a small 6 cm long thorned blackberry stem which had broken off and lodged in its vagina. The lodged stem produced an acute inflammatory response of the vagina which resulted in the formation of an abscess which expressed copious amounts of purulent exudate. Swabs of the exudate revealed a heavy mix of bacteria, including gram positive cocci and faint staining gram negative bacilli. The young female also had a small abscess in the perineal region. A juvenile male otter (RAG-169) taken at the Fall Creek Fish Hatchery on the Santiam River of Oregon had a large draining abscess at the base of the tail above the anus that measured 4 cm in diameter and ~2 cm deep. The abscess appeared to be associated with the anal gland and contained a light creamy yellow exudate. Juvenile female otter RAG-016 from the lower Siuslaw River, OR had a cyst on its left ovary. Juvenile male otter RAG-018 also from the lower Siuslaw River had a small clear cyst on its right thyroid gland. A two year old male otter (RAG-063), trapped from a small suburban lake south of Hillsboro, Oregon, had a pale tan liver. The trapper said the otter was observed several times eating dog food, which contains fats not normally consumed by otters and could possibly account for the liver's color. A nutmeg colored liver was noted in an 8 year old otter (RAG-221) from Petroleum Creek, WA, who was extremely obese. The color may be indicative of either congestive heart or liver issues. An eleven year old male otter (RAG-256) from Peterson Lake, WA had a

perforated upper small intestine, apparently from what appeared to be a long fish rib bone. The perforation had healed and the rib bone immobilized outside and alongside the intestines. The mass surrounding the bone was hard and mineralized. Finally, a seven year old male otter (RAG-245) collected from the Skagit River of western Washington had bilateral nephrolithiasis and ureteral hypertrophy (Grove et al. 2003). The uroliths were 97% uric acid and 3% protein. The ureters were enlarged due to marked hypertrophy of smooth muscle plus dilation of the lumen. Fusion of the major calyces into a single ureteral lumen was several cm distal to that of unaffected male otters used as histopathologic control specimens. The central nervous system was not examined on any of the otter specimens. No external deformities of the toes were noted. No deformities were noted for the male reproductive tract. An example of a normal male otter urogenital tract is shown in Figure 11. The testes for all ages of male otters were always located in the region of the scrotum, with testes hypertrophy apparent after mid-December.

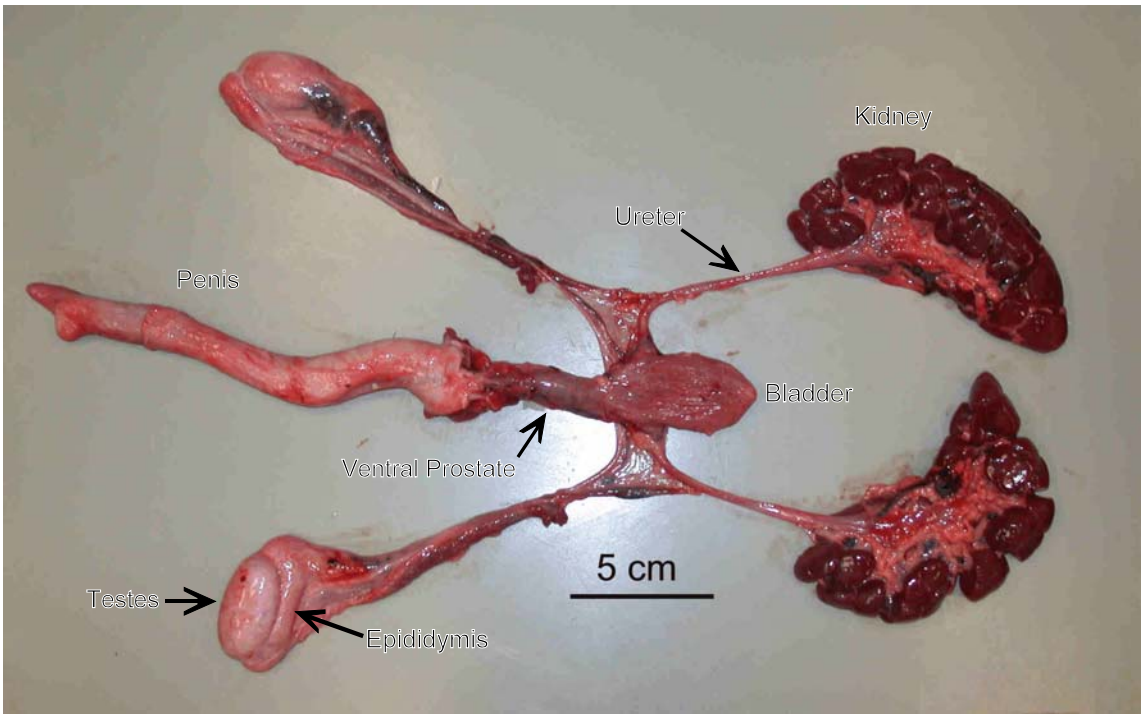


Figure 11. Normal river otter male urogenital tract.

Histopathology Findings

Histology slides were prepared for all of the frozen river otters collected in 1994-95. Mild granulomatous pneumonia with multi-focal PAS positive fungal organisms within the center of alveolar inflammatory foci were found in river otters OT-01 (five year old male) and OT-02 (three year old male), both collected at river mile 73.1 of the Columbia River. The pyogranulomatous abscess and cellulitis for otter OT-37 had an operculated parasite ova with a brown cell wall associated with the abscess. In the remaining otters, no significant histologic changes, besides varying degrees of postmortem autolysis and freezing artifacts, were observed in the kidney, liver, lungs, mesenteric lymph node, spleen, thymus, thyroid gland, testes, ovary or uterus.

Histological slides were prepared of tissues from 30 unfrozen and 9 live-trapped river otters collected from 1996-99. Most of the livers examined had a few hepatic portals rimmed by a few lymphocytes and plasma cells. This is usually noted as a abnormality, but the change was so minor and so common as to be considered “normal variation.” However, a few significant observations are noted in Table 2. The most prevalent observation in the otter tissues examined was some form of histologic change in the lungs, of which 16 of 39 (41%) otters evaluated were affected. Thirty-two percent (8 of 25) of the otters from the coastal areas of Oregon and Washington had lung histologic changes compared to 57% (8 of 14) of the otters from the LCR and WB. However, the incidence of histologic changes in the lung was independent of location (Chi-Square P = 0.126, n = 39). These histologic changes were probably associated with water aspiration and not considered significant pathologies. Otter RAG-003 had a single intra bronchiolar fluke with local infiltration of macrophages, eosinophils, and some lymphocytic perivascular cuffing. Five otters (RAG-005, 007, 015, 030, 031) had pulmonary granulomas containing some type of foreign material, with two containing a circular basophilic acellular structure lacking a capsule (RAG-030) or a spherical organism with a well defined shell (RAG-005). A four year-old male from Siltcoos Lake, OR (RAG-007) also had multiple pulmonary

granulomas associated with diffuse pulmonary congestion and edema. Spheres of condensed intraalveolar protein with varying degrees of mild cellular reaction was evidenced by associations of macrophages, lymphocytes, neutrophils and eosinophils for otters RAG-017, 019, 026, 027, 028, 029, RAG98-02, RAG99-01, RAG99-02 and RAG99-06 (Table 2). A hemorrhagic lymph node associated with the lung tissue from otter RAG99-06 had neutrophils and macrophages in the paratrabecular sinuses, with a few bacterial colonies in the subcapsular sinus.

Four otters had minimal colloid in the thyroid, with another (RAG-004) having poor colloid production. All five otters were from coastal lakes and rivers of Oregon (Table 2). Multi focal adrenocortical hemorrhage was noted in three of five otters (RAG-023, 024 and RAG98-04) with adrenal gland findings. Otter RAG-025 had focal telagiectasis (dilated sinusoids) in the adrenal cortex. Otter RAG-005 had mild to moderate lymphocytic infiltrate along the corticomedullary junction of the adrenal gland, but a lot of functional gland persisted and no cellular necrosis was evident. Livers of two otters (RAG-014, 021) had multiple foci of hepatocellular vacuolation consistent with glycogen deposition and another otter (RAG-010) had mild periportal lymphoplasmacytic hepatitis. A single lymphoplasmacytic perivascular cuff was found in the kidney of otter RAG-024. Though not significant, otter RAG-004 had a single focus of interstitial nephritis. Otter RAG98-04 had nephrosis associated with granular eosinophilic debris and necrotic epithelial cells in many tubular lamina and a flattening of tubular epithelial cells. A third otter (RAG-010) had moderate pyogranulomatous tubulointerstitial nephritis associated with many plasma cells. This finding was severe enough to potentially cause clinical disease. Special staining revealed the nephritis to be positive for spirochetes, which is strongly suggestive of Leptospira. There were two findings involving the pancreas, a mild lymphoplasmacytic infiltrate around the large pancreatic ducts of one otter (RAG-011), and another with pancreatic interstitial hemorrhage (RAG-024). One juvenile otter (RAG-010) was observed with a thymic hemorrhage. Otter RAG-014 had a gastric cyst where the ulceration of the lining included many plasma

Table 2. Histological findings for live-trapped and unfrozen river otters collected from Oregon and Washington, 1996-99.

Location	Age	Adrenal	Kidney	Liver	Lung	Pancreas	Thymus	Thyroid	Epididymis	Testicle	Uterus	Ovary
Coastal Lakes and Rivers												
Males												
RAG-009 (Siltcoos Lake, OR)	0										-	-
RAG-010 (Siltcoos Lake, OR)	0		X	X			X				-	-
RAG-012 (Clearwater R., WA)	0										-	-
RAG-017 (Siuslaw R., OR)	0				X						-	-
RAG-018 (Siuslaw R., OR)	0										-	-
RAG-002 (Siltcoos Lake, OR)	1							X			-	-
RAG-003 (Siltcoos Lake, OR)	1				X			X			-	-
RAG-024 (Coquille R., OR)	1	X	X								-	-
RAG99-01 (Woahink LK, OR	1				X				X		-	-
RAG-008 (Siltcoos Lake, OR)	2								X		-	-
RAG-011 (Dickey R., WA)	2					X			X		-	-
RAG-022 (Nehalem R., WA)	2										-	-
RAG-023 (Coquille R., OR)	2	X									-	-
RAG-001 (Yaquina R., OR)	4										-	-
RAG-007 (Siltcoos Lake, OR)	4				X						-	-
RAG98-02 (Metolius R., OR)	5				X						-	-
RAG-020 (Siuslaw R., OR)	6										-	-
RAG-004 (Siltcoos Lake, OR)	9		X					X			-	-
RAG-021 (Chehalis R., WA	9			X							-	-
RAG-014 (Siuslaw R., OR)	12			X		X		X			-	-
Females												
RAG-013 (Clearwater R., WA)	0								-	-		
RAG-016 (Siuslaw R., OR)	0							X	-	-		
RAG-015 (Siuslaw R., OR)	2				X				-	-		
RAG-031 (Woahink LK, OR)	3				X				-	-	X	
RAG-019 (Siuslaw R., OR)	5				X				-	-		X
Lower Columbia River and Willamette River Basin												
Males												
RAG98-01 (Portland, OR)	0										-	-
RAG98-04 (Sauvie Is., OR	0	X	X								-	-
RAG-005 (Creswell, OR)	1	X			X						-	-
RAG-025 (Portland, OR)	1	X							X		-	-

Table 2. Continued.

Location	Age	Adrenal	Kidney	Liver	Lung	Pancreas	Thymus	Thyroid	Epididymis	Testicle	Uterus	Ovary
Lower Columbia River and Willamette River Basin												
Males												
RAG99-03 (Portland, OR)	1								X	X	-	-
RAG99-06 (Chinook L., OR)	1				X				X		-	-
RAG-029 (Scappoose, OR)	3				X				X		-	-
RAG-026 (Albany, OR)	4				X						-	-
RAG-027 (Albany, OR)	4			X	X				X		-	-
RAG-028 (Albany, OR)	4				X				X		-	-
RAG-030 (Albany, OR)	4				X						-	-
Females												
RAG-085 (Newberg, OR)	1								-	-	X	
RAG99-04 (Portland, OR)	1								-	-		
RAG99-02 (Chinook L., OR)	1				X				-	-		

cells, hemosiderin laden macrophages and multinucleate giant cells. Another otter (RAG-019) also had a gastric nodule of transmural eosinophilic inflammation with fewer mast cells and plasma cells. No ulcerous tissue was observed and although no parasite was present, the reaction is typical of that directed at endoparasites.

The most common histological finding of male river otter reproductive organs was a mild to moderate lymphoplasmacytic interstitial epididymitis found in nine otters, with ages ranging from yearlings to four years. Occurrence of the epididymitis was 20% (3 of 15) for the coastal male otters and 56% (6 of 9) for male otters from LCR and WB. The incidence of epididymitis was dependent on location (Chi-Square P = 0.0736, n = 24), with a higher frequency of findings occurring on the LCR and WB. One of the otters (RAG-027) also had lymphoplasmacytic infiltrate where the ductus deferens penetrates through the muscular wall of the urethra/prostate. Though a yearling otter RAG99-03 had

active spermatogenesis, the epididymis contained only degenerate epithelial cells. A few spermatid giant cells were also visible in the spermatic tubules of the testis. Unidentified tissue associated with the epididymis of yearling otter RAG99-01 was suspected of being remnant Müllerian duct as the lumen was lined by low columnar non-ciliated epithelium with poorly developed tubular glands. The prostate section of the urethra of RAG99-01 also had no prostate tissue associated it. Otter RAG-021 (9 year old male) exhibited prostatic hyperplasia with increased amounts of interstitial collagen and a small number of plasma cells, lymphocytes and eosinophils. A female otter (RAG-031) had mild uterine neutrophilic endometritis with the oviducts unaffected. Finally, female otter RAG99-02 that had the aforementioned blackberry stem lodged in its vagina had epidermititis with nuclear inclusions in the vulvar/vaginal epithelial cells. Some of these cells exhibited karyomegaly, suggesting a possible viral etiology. Epithelial erosion occurred with large numbers of neutrophils in the epidermis and subjacent dermis, with some areas predominated by lymphocytes, plasma cells and some eosinophils. The perineal abscess of the same female consisted of multiple epidermal inclusion cysts.

Three frozen liver samples suspected of lipidosis were submitted for histology. Only the liver sample from RAG-221 was found to have mild to moderate lipidosis and the nutmeg color described earlier was a result of autolysis. The lipidosis may be due to the obese nature of the eight year old otter described earlier.

The calcified mass found along the outside of the upper small intestine of otter RAG-256 was a chronic focus of fibrosis with mineral deposits in the tissue. Some of the tissue included necrotic fat (which often becomes mineralized) and what may be the outline of a very thick cuticle, suggestive of an encysted tapeworm.

Reproductive organ histology slides prepared from the frozen tissue of 194 male river otters collected in 1996-97 consisted of 53 juveniles, 32 yearlings and 109 adults. Combined with the histology data from the unfrozen and live-

trapped otters of 1996-99 and the frozen otter carcasses of 1994-95, the total number of males was 63 juveniles, 40 yearlings and 135 adults. Juvenile males showed no sign of seminiferous tubule enlargement or active spermatogenesis during the collection periods (Figure 12), except for RAG-055. Otter RAG-055 may be a yearling which had been born late in the spring and had yet to form its first cementum annulus of the canine tooth used to age the otter. Seminiferous tubule size and tubule cellular proliferation was found to increase throughout the collection period for both yearlings and adults (Figure 12). Spermatogenesis was apparent in some of the adult and yearling males as early as 1 December, with spermatogenesis observed in almost all yearling and adult males after 1 January (Figure 13). These observations do not evaluate the extent of spermatogenesis occurring, only that spermatogenesis was apparent.

Hematology and Blood Chemistry Findings

All live-trapped river otters were bled and necropsied within 12 hours of capture, except for otter RAG-025 which was held for more than 24 hours. Hematologic data for the 9 live-trapped otters are shown in Table 3 and are compared with otter data reported by Tocidlowski et al. (2000) from otters live-trapped in North Carolina (Table 4). The North Carolina otters were fasted for 12 hours prior to blood collection. Yearling otters from this study were considered adults for complete blood count comparisons to data reported in Tocidlowski et al. (2000). In general, erythrocyte values were within the range reported by Tocidlowski et al. (2000) though at the upper end. Platelet counts were considered adequate for otters in this study. Leukocyte numbers were elevated in two otters (RAG-025, RAG99-02) in the total white blood cell count and for both absolute neutrophils and monocytes, while also having significantly lower lymphocyte numbers for these two otters. The juvenile female mentioned above (RAG99-02) which had the vaginal infection resulting from the lodgement of the blackberry stem, may be responsible for the elevated neutrophils and monocytes and the depletion of the lymphocytes. Elevated neutrophil and monocyte

numbers associated with depressed lymphocyte numbers of the yearling male otter (RAG-025) may be due to prolonged stress as a result of being held for a longer period of time. Dogs can exhibit this when under long periods of constant stress.

Mean blood chemistry concentrations were determined by combining all ages as no age-related differences were apparent. Individual blood chemistry concentrations for calcium, chloride, phosphorus and sodium were within the range reported by Tocidlowski et al. (2000), with similar though slightly higher mean concentrations from this study (8.8 mg/dl, 118 mEq/L, 6.2 mg/dl,158 mEq/L, respectively) for study otters. Mean potassium in this study was less than the 4.2 mEq/L reported by Tocidlowski et al. (2000). Concentrations of calcium, phosphorus, sodium and potassium were slightly higher for RAG-025 which might have resulted from being held longer than 24 hours prior to blood collection. Alanine aminotransferase (ALT) was quite variable, but the mean concentration was lower than reported by Tocidlowski et al. (2000). Mean alkaline phosphatase was 34% higher than that reported at 129 IU/L, with RAG99-02 having a very high concentration of 634 IU/L. Mean cholesterol (170 mg/dl), total protein (7.4 g/dl), blood urea nitrogen (BUN) (42.7 mg/dl), and glucose (149 mg/dl) were higher than values reported by Tocidlowski et al. (2000). Mean creatinine was slightly lower than reported. Otter RAG-025 had the lowest individual concentration of blood glucose, which may have been due to being held longer than 24 hours without eating and possibly capture stress. Alkaline phosphatase and BUN were higher in concentration for the RAG99-02 juvenile female than the other live-trapped otters in this study. The elevated BUN may have resulted from the vaginal infection, possibly due to the female's inability to acquire enough food and causing protein wasting to occur. The high alkaline phosphatase concentration is less understood.

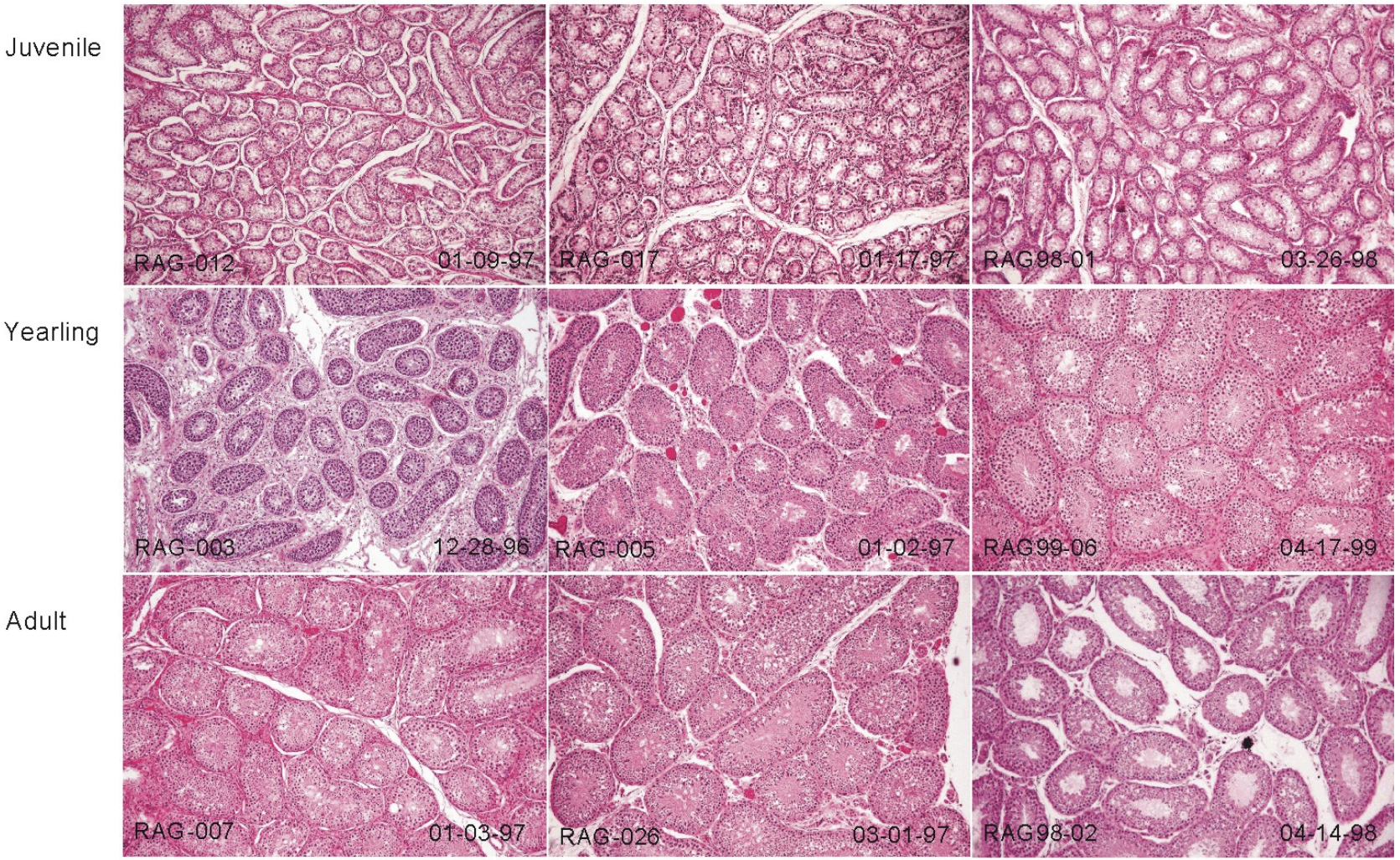


Figure 12. Testes histology images at 5X power from the 3 age groups of river otter males collected from Oregon and Washington throughout the trapping season. Note the size of the seminiferous tubules.

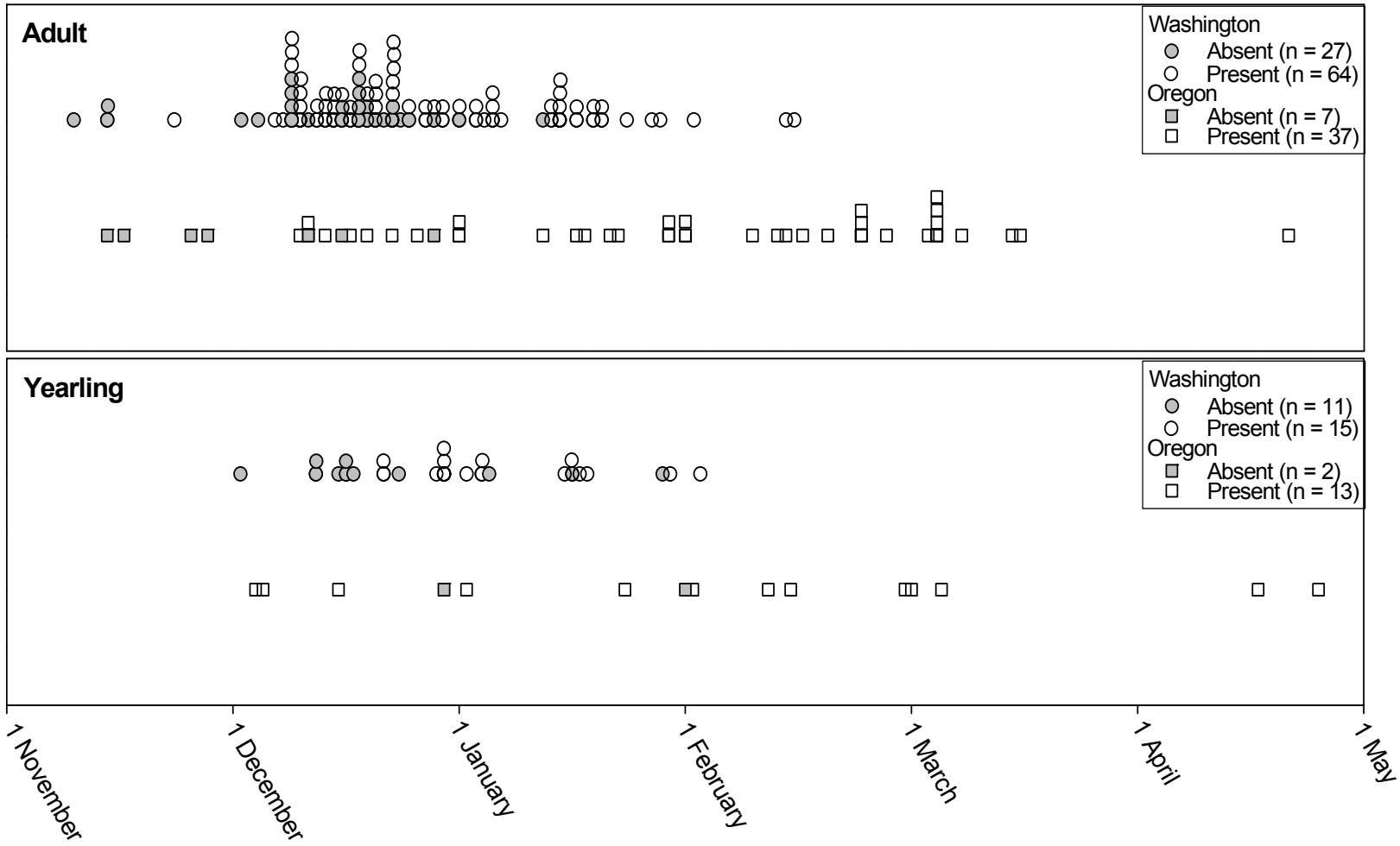


Figure 13. The relationship between trap date and whether spermatogenesis was absent or present in the testes of adult and yearling river otter males collected from Oregon and Washington, 1994-99.

Table 3. Hematology and serum chemistry values for river otters live-trapped from Oregon, 1996-99.

		Study Otters								
Parameter	Units	RAG-025	RAG-031	RAG-085	RAG98-01	RAG98-02	RAG99-01	RAG99-02	RAG99-03	RAG99-04
Age	Years	1	3	1	0	5	1	0	1	1
Sex		male	female	male	male	male	male	female	male	female
WBC	$x10^6$/µl	38.2	10.0	16.5	19.2	22.4	10.3	25.1	14.9	13.2
RBC	$x10^3$/µl	11.7	11.7	11.0	10.6	8.56	11.7	11.5	9.57	10.4
Hemoglobin	g/dl	19.4	17.5	17.0	16.9	13.7	17.7	18.3	14.6	15.3
Hematocrit	%	60.6	54.6	50.7	53.2	42.8	57.2	58.6	49.0	52.1
Packed Cell Volume	%	55.0	50.8	47.0	48.0	39.0	52.0	55.0	43.0	43.0
Plasma Protein	g/dl	10.1	8.0	7.5	8.8	7.0	7.8	8.7	8.0	8.0
Fibrinogen	mg/dl	300								
MCV	fl	51.9	46.7	46.3	50.0	50.0	49.0	51.0	51.2	50.0
MCH	pg	16.6	15.0	15.5	15.9	16.0	15.2	15.9	15.3	14.7
MCHC	g/dl	32.0	32.1	33.5	31.8	32.0	30.9	31.2	29.8	29.4
Protein:fibrinogen ratio		33.7	80.0	25.0			26.0			
Platelets		Ade[a]	Ade	Ade	Ade	Ade	Ade	Ade	Ade	Ade
Neutrophils	#/µl	Oca[b]						Oca[c]		
Segmented	#/µl	32,088	7,900	15,180	17,472	19,040	6,695	20,582	13,410	10,956
Bands	#/µl	382						1004		
Lymphocytes	#/µl	382	1,800	1,320	1,344	3,360	2,060	251	1,192	1,584
Monocytes	#/µl	5,348	300		192		1,236	3,263	298	660
Eosinophils	#/µl	Oca			192		309			
Calcium	mg/dl	9.1	8.5	8.7	9.2	8.3	8.8	8.5	9.0	9.1

Table 3. Continued.

		Study Otters								
Parameter	Units	RAG-025	RAG-031	RAG-085	RAG98-01	RAG98-02	RAG99-01	RAG99-02	RAG99-03	RAG99-04
Phosphorus	mg/dl	7.4	5.5	6.7	6.8	8.0	3.9	5.6	6.1	6.2
Sodium	mEq/L	170	161	155	163	158	154	156	156	149
Potassium	mEq/L	4.8	4.1	4.0	4.5	4.1	4.3	3.6	4.3	4.3
Chloride	mEq/L	123	123	116	121	118	114	118	119	112
ALT	IU/L	175	161	145	101	129	79.0	181	94	123
Alkaline Phosphatase	IU/L	85.0	177	130	73.0	130	97.0	634	87	199
Total Bilirubin	mg/dl	0.2	0.3	0.2	0.1	0.2	0.2	0.3	0.2	0.2
Cholesterol	mg/dl	113	171	168	231	127	147	167	211	192
GGT	IU/L						9.0	44.0	16	56
Total Protein	g/dl	8.8	7.1	6.8	8.0	6.5	7.1	7.7	7.4	7.6
Globulins	g/dl	4.9	3.8	3.3	4.1	3.5	3.7	4.4	4.0	4.3
Albumins	g/dl	3.9	3.3	3.5	3.9	3.0	3.4	3.3	3.4	3.3
BUN	mg/dl	36.6	37.3	25.3	32.0	57.0	27.0	84.0	42.0	43.0
Creatinine	mg/dl	0.3	0.5	0.4	0.3	0.3	0.3	0.2	0.3	0.2
Glucose	mg/dl	92	166	103	209	221	126	129	127	169

[a] Ade = adequate.

[b] Oca = occasional neutrophils with toxic granules, occasional reactive monocytes.

[c] Oca = occasional neutrophils with toxic granules, vacuoles, and/or Dohle bodies.

WBC = white blood cell count, RBC = red blood cell count, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, ALT = alanine aminotrnferase, GGT = γ-glutamyl transferase, BUN = blood urea nitrogen

Table 4. Hematology and serum chemistry values for river otters from Tocidlowski et al. 2000.

			Adult		Juvenile		
Parameter	Units	n	Median	Range	n	Median	Range
WBC	$x10^3/\mu l$	132	11.3	4.7-33.2	23	7.1	3.2-13.1
RBC	$x10^6/\mu l$	132	11.0	6.1-14.5	23	10.1	7.46-13.7
Hemoglobin	g/dl	132	15.1	10.4-19.0	23	13.5	11.4-17.9
Hematocrit	%	132	47.6	32.2-60.8	23	43.4	35.1-57.6
MCV	fl	132	43.3	38.3-49.0	23	42.8	40.6-48.9
MCH	pg	132	13.7	11.3-15.8	23	13.8	12.9-16.0
MCHC	g/dl	132	31.4	27.8-39.2	23	32.0	28.4-36.2
Platelets		132	565	298-931	23	561	394-834
Neutrophils	#/µl	132	8879	3003-28220	23	5084	1778-10962
Bands	#/µl	132	94	0-486	23	62	0-232
Lymphocytes	#/µl	132	1254	123-4950	23	1183	441-3200
Monocytes	#/µl	132	452	52-2380	23	224	28-963
Eosinophils	#/µl	132	312	0-1833	23	205	0-1092
Basophils	#/µl	132	88	0-219	23	58	0-63
Calcium	mg/dl	50	8.4	6.8-10.0			
Phosphorus	mg/dl	50	5.8	3.2-8.3			
Sodium	mEq/L	50	152	136-158			
Potassium	mEq/L	50	4.4	3.5-5.3			
Chloride	mEq/L	50	113	94-121			
ALT	IU/L	50	194	46-990			
Alkaline Phosphatase	IU/L	50	85	29-282			
Total Bilirubin	mg/dl	50	0.2	0.1-0.5			
Cholesterol	mg/dl	29	152	63-279			
GGT	IU/L	29	19	8-38			
Total Protein	g/dl	50	7.3	5.7-9.0			
Globulins	g/dl	50	4.0	2.9-5.8			
Albumins	g/dl	50	3.3	2.4-4.1			
BUN	mg/dl	50	31.0	17-56			
Creatinine	mg/dl	50	0.5	0.4-0.8			
Glucose	mg/dl	50	130	56-225			

WBC = white blood cell count, RBC = red blood cell count, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, ALT = alanine aminotrnferase, GGT = γ-glutamyl transferase, BUN = blood urea nitrogen.

Morphometrics

River otter mean morphometric measurements are shown by age and state in Table 5. Two-way ANOVAs using the independent variables of age and state revealed that river otter males were generally not significantly different in size between Oregon and Washington. However, some morphometric differences between states were detected. Yearling male otters from Washington were heavier (mean body mass) than yearling otters from Oregon ($P = 0.02$). The thymus mass for adult males from Washington was heavier than for adult males from Oregon ($P = 0.01$). Mean lung mass of both yearling ($P = 0.003$) and adult ($P = 0.002$) males from Washington was heavier than yearling and adult males from Oregon. Mean thyroid ($P = 0.0002$) mass of adult males from Washington was also heavier than adult males from Oregon. Oregon adult males, though, had heavier mean pancreas mass ($P = 0.002$), while both juvenile ($P = 0.02$) and adult males from Oregon ($P = 0.009$) had heavier mean adrenal masses than juvenile and adult males from Washington. Mean testes mass of yearling ($P = 0.004$) and adult ($P < 0.0001$) males from Oregon were heavier than yearling and adult males from Washington. Mean testes mass was also heavier for Oregon juveniles compared to juveniles from Washington, though not significant ($P = 0.153$). Juvenile, yearling and adult males from Oregon had heavier mean epididymis ($P = 0.03$, $P = 0.006$, $P < 0.0001$, respectively) and gonad ($P = 0.045$, $P = 0.02$, $P < 0.0001$, respectively) mass than juvenile, yearling and adult males from Washington. Mean baculum mass and length were also heavier and longer for the three Oregon age groups when compared to Washington, though not significant. Finally, mean prostate mass ($P = 0.03$) was heavier for adult males from Oregon than adult males from Washington. When data from both states were combined, means of juvenile male otters were significantly smaller for all morphometric measurements compared to both yearlings and adults ($P \leq 0.0033$) except for thymus mass which was larger ($P<0.0001$) (Table 6). Yearling males were generally smaller

than adult males except for (though not significant) mean total length, tail length, femur length, humerus length, thymus mass, spleen mass and adrenal mass. Significant differences ($P < 0.0001$) were found with kidney, epididymis, gonad, prostate, baculum masses and baculum length between all three age groups, with juveniles the smallest, followed by yearlings and then adults. Otter thymus mass was significantly correlated with age in an inverse manner, with thymus mass reduction gradual over time (Figure 14).

Of those morphometric differences found within age groups, only adult male gonadal tissues (testis, epididymis and gonad) had significant correlations relating to seasonal effects associated with reproductive hypertrophy (Figures 15 and 16). Juvenile males from Oregon and Washington showed no seasonal response to gonadal hypertrophy, while yearlings revealed a small though not significant response to seasonal hypertrophy (Figure 15). Trap date accounted for less than 50% of the variability in adult male testes mass for both Oregon ($r^2 = 0.496$) and Washington ($r^2 = 0.232$) were less than 50%. However, the data for each state resulted in parallel lines (Figure 16), which suggests that adult males from Oregon come into breeding condition about 13 days earlier than adult males from Washington.

Mean juvenile (<1 year old) male baculum mass and length were significantly lighter and shorter when compared to the other age groups (Table 7). Maximum baculum mass and length appears to occur after the age of three. Mean juvenile male femur, humerus and scapula mass was lighter ($P < 0.0001$) compared to the other age groups (Table 8). Mean juvenile femur length was significantly shorter than the other age means, except age seven (only 7 animals). Juvenile mean humerus length was also shorter than the other age group means, but significant only for age groups six and above eight. Graphically, otter baculums reach maximum length after the age of three, while

Table 5. Mean morphometric comparisons between juvenile, yearling and adult river otter males collected from Oregon and Washington, 1994-99. OR = Oregon, WA = Washington and Comb. = combined.

	Juvenile			Yearling			Adult		
Parameter	OR[a]	WA[b]	Comb.[c]	OR[d]	WA[e]	Comb.[f]	OR[g]	WA[h]	Comb.[i]
n	18	46	65	19	28	46	50	93	143
Total Length (cm)	108 A	107 A	107	115 A	118 A	117	115 A	116 A	116
Tail Length (cm)	44.2 A	45.2 A	44.9	48.3 A	49.0 A	48.7	48.2 A	48.5 A	48.4
Neck Circumference (cm)	25.5 A	26.0 A	26.0	28.3 A	28.7 A	28.5	28.9 A	29.3 A	29.1
Body Mass (kg)	6.38 A	6.66 A	6.58	**7.74 B**	**8.66 A**		8.55 A	8.98 A	8.83
Liver Mass (g)	391 A	385 A	387	446 A	505 A	482	519 A	485 A	497
Thymus Mass (g)	10.4 A	12.1 A	11.7	7.49 A	9.24 A	8.56	**5.29 B**	**7.36 A**	
Heart Mass (g)	58.6 A	59.1 A	59.0	71.4 A	77.3 A	75.0	80.2 A	81.0 A	80.7
Lung Mass (g)	147 A	157 A	154	**152 B**	**196 A**		**185 B**	**201 A**	
Spleen Mass (g)	40.5 A	35.0 A	36.5	52.1 A	52.3 A	52.2	48.2 A	47.6 A	47.8
Pancreas Mass (g)	29.7 A	31.7 A	31.1	35.9 A	36.8 A	36.4	**41.4 A**	**35.0 B**	
Thyroid Mass (g)	0.64 A	0.76 A	0.73	0.76 A	0.91 A	0.85	**0.81 B**	**0.98 A**	
Kidney Mass (g)	86.7 A	83.0 A	84.2	91.8 A	103 A	98.5	106 A	108 A	107
Adrenal Mass (g)	**1.02 A**	**0.87 B**	0.91	1.14 A	1.05 A	1.08	**1.12 A**	**0.96 B**	
Testes Mass (g)	2.03 A	1.73 A	1.81	**19.8 A**	**13.5 B**		**22.0 A**	**15.9 B**	
Epididymis Mass (g)	**2.99 A**	**2.32 B**		**12.3 A**	**9.50 B**		**14.3 A**	**11.2 B**	
Gonad Mass (g)	**4.95 A**	**4.01 B**		**30.5 A**	**23.7 B**		**38.1 A**	**27.3 B**	
Prostate Mass (g)	1.23 A	1.26 A	1.25	1.94 A	1.80 A	1.85	**2.73 A**	**2.46 B**	
Baculum Mass (g)	2.72 A	2.58 A	2.62	6.29 A	5.78 A	5.98	7.01 A	6.78 A	6.86
Baculum Length (mm)	82.6 A	81.2 A	81.6	97.1 A	94.5 A	95.5	98.4 A	97.7 A	98.0
Femur Mass (g)	9.03 A	8.99 A	9.00	10.8 A	11.1 A	11.0	11.6 A	11.5 A	11.5
Femur Length (mm)	77.7 A	77.7 A	77.7	82.3 A	83.4 A	83.0	83.1 A	82.6 A	82.7
Humerus Mass (g)	9.81 A	9.58 A	9.65	11.9 A	12.1 A	12.0	12.8 A	12.6 A	12.7
Humerus Length (mm)	77.4 A	77.1 A	77.1	80.8 A	82.7 A	81.9	81.8 A	81.4 A	81.5
Scapula Mass (g)	4.31 A	4.54 A	4.48	6.00 A	6.06 A	6.04	6.25 A	6.28 A	6.27

Table 5. Continued.

One way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows of categories showing the same letters are not significantly different. Means combined if not significantly different from one another.

[a] N=17 for liver mass, lung mass, spleen mass, adrenal mass. N=16 for testes mass, epididymis mass femur mass, femur length, humerus mass and humerus length. N=15 for heart mass and prostate mass. N=14 for scapula mass.

[b] N=45 for total length, tail length, body mass, spleen mass and gonad mass. N=43 for baculum mass and baculum length. N=42 for heart mass, testes mass, epididymis mass, femur mass, femur length and scapula mass. N=41 for prostate mass. N=40 for humerus mass and humerus length.

[c] N=64 for total length, tail length, body mass, liver mass, lung mass, and adrenal mass. N=63 for spleen mass. N=61 for baculum mass and baculum length. N=59 for testes mass, femur mass and femur length. N=58 for heart mass. N=57 for prostate mass, humerus mass, humerus length and scapula mass.

[d] N=18 for neck circumference, body mass, liver mass, thymus mass, lung mass, spleen mass, pancreas mass, thyroid mass, kidney mass, adrenal mass, gonad mass, baculum mass and baculum length. N=15 for heart mass, testes mass, epididymis mass, femur mass, femur length, humerus mass, humerus length and scapula mass. N=13 for prostate mass.

[e] N=27 for body mass. N=23 for heart mass, testes mass, epididymis mass, prostate mass, femur mass, femur length, and scapula mass. N=22 for humerus mass and humerus length.

[f] N=45 for neck circumference, liver mass, thymus mass, spleen mass, pancreas mass, thyroid mass, kidney mass, and adrenal mass. N=37 for heart mass, femur mass, femur length and scapula mass. N=36 for humerus mass and humerus length. N=35 for prostate mass.

[g] N=49 for total length, liver mass, thymus mass, spleen mass, pancreas mass, baculum mass and baculum length. N=48 for tail length. N=47 for gonad mass. N=43 for heart mass, prostate mass, femur mass and femur length. N=42 for testes mass and scapula mass. N=41 for epididymis mass, humerus mass and humerus length.

[h] N=92 for neck circumference, pancreas mass and thyroid mass. N=90 for total length, tail length and body mass. N=87 for heart mass, testes mass, epididymis mass and prostate mass. N=86 for femur mass, femur length and scapula mass. N=85 for humerus mass and humerus length.

[i] N=142 for neck circumference, liver mass, spleen mass, baculum mass and baculum length. N=140 for body mass. N=139 for total length. N=138 for tail length. N=130 for heart mass. N=129 for femur mass and femur length. N=128 for scapula mass. N=126 for humerus mass and humerus length.

Table 6. Mean morphometric comparisons between juvenile, yearling and adult male river otters collected 1994-99, with states combined.

	Juvenile		Yearling		Adult		
Parameter	n	Mean	n	Mean	n	Mean	P
Total Length (cm)	63	107 B	47	117 A	139	116 A	<0.0001
Tail Length (cm)	63	44.9 B	47	48.7 A	138	48.4 A	<0.0001
Neck Circumference (cm)	64	26.0 B	46	28.5 A	142	29.1 A	<0.0001
Body Mass (kg)	63	6.58 B	45	8.29 A	140	8.83 A	<0.0001
Liver Mass (g)	63	387 B	46	482 A	142	497 A	<0.0001
Thymus Mass (g)	64	11.7 A	46	8.56 B	142	6.65 B	<0.0001
Heart Mass (g)	57	59.0 B	38	75.0 A	130	80.7 A	<0.0001
Lung Mass (g)	63	154 B	46	179 A	143	196 A	<0.0001
Spleen Mass (g)	62	3.65 B	46	52.2 A	142	47.8 A	<0.0001
Pancreas Mass (g)	64	31.1 B	46	36.4 A	141	37.2 A	<0.0001
Thyroid Mass (g)	64	0.73 B	46	0.85 AB	142	0.92 A	0.0003
Kidney Mass (g)	64	84.2 C	46	98.5 B	143	107 A	<0.0001
Adrenal Mass (g)	63	0.91 B	46	1.08 A	143	0.91 AB	0.0033
Testes Mass (g)	58	1.81 B	38	16.0 A	129	17.9 A	<0.0001
Epididymis Mass (g)	58	2.50 C	38	10.6 B	128	12.2 A	<0.0001
Gonad Mass (g)	63	4.28 C	46	26.4 B	140	30.9 A	<0.0001
Prostate Mass (g)	56	1.25 C	36	1.85 B	130	2.55 A	<0.0001
Baculum Mass (g)	61	2.62 C	46	5.98 B	142	6.86 A	<0.0001
Baculum Length (mm)	61	81.6 C	46	95.5 B	142	98.0 A	<0.0001
Femur Mass (g)	58	9.00 B	38	11.0 A	129	11.5 A	<0.0001
Femur Length (mm)	58	77.7 B	38	83.0 A	129	82.7 A	<0.0001
Humerus Mass (g)	56	9.65 B	37	12.0 A	126	12.7 A	<0.0001
Humerus Length (mm)	56	77.1 B	37	81.9 A	126	81.5 A	<0.0001
Scapula Mass (g)	56	4.48 B	38	6.04 A	128	6.27 A	<0.0001

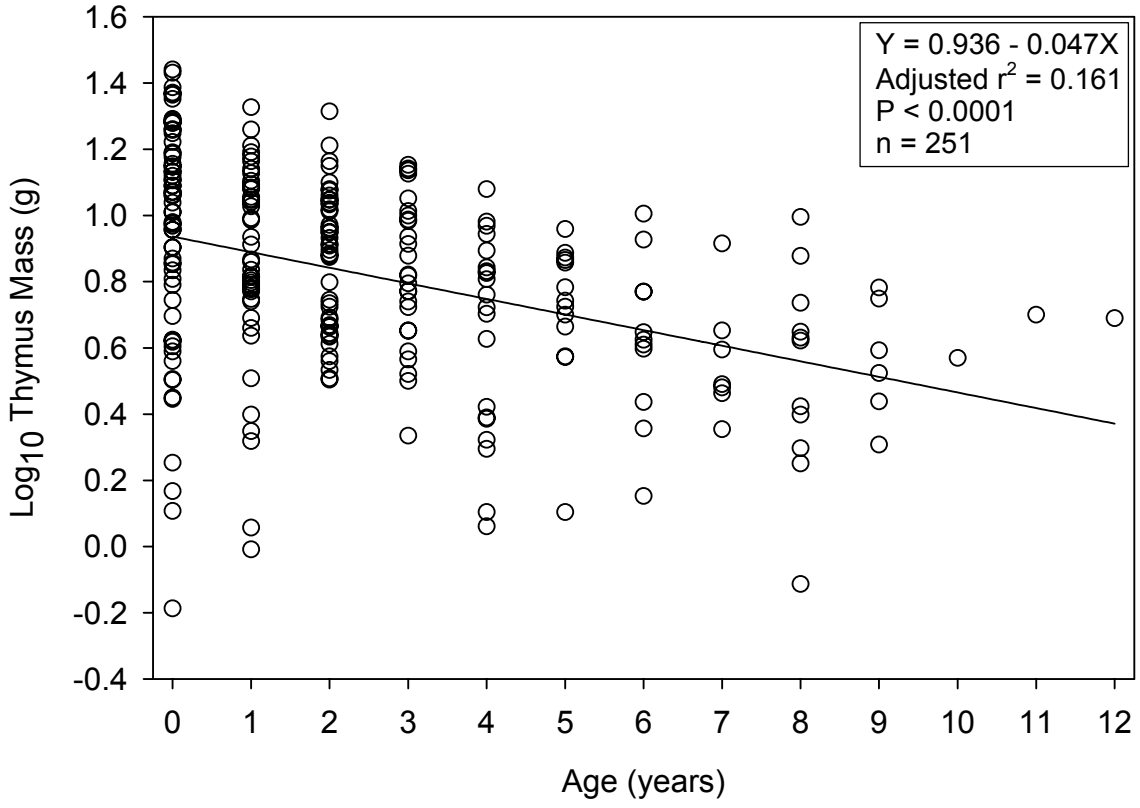


Figure 14. Relationship between age and $\log_{10}$ thymus mass (g) for river otter males collected from Oregon and Washington, 1994-99.

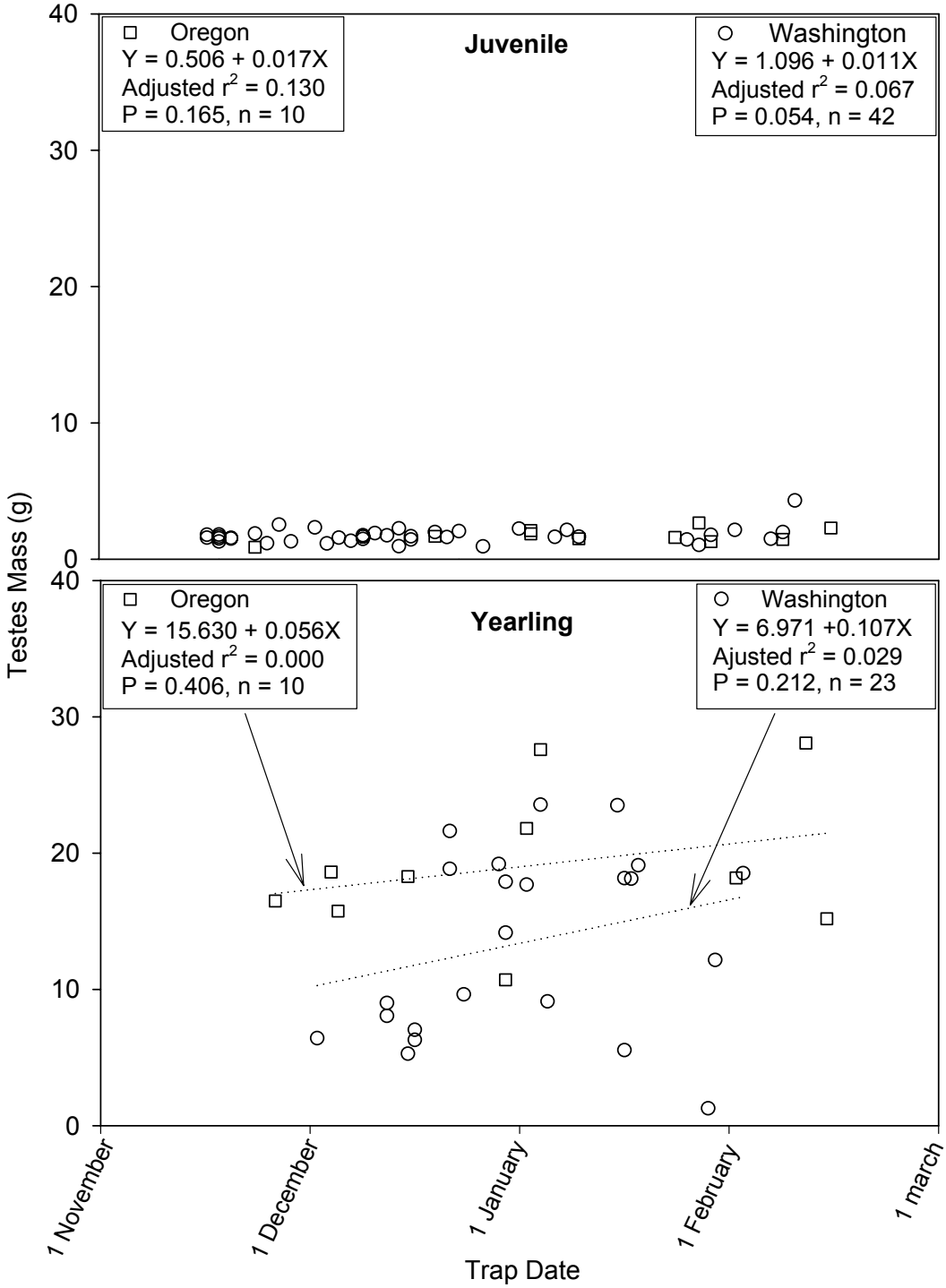


Figure 15. Relationship between testes mass (g) and trap date (by state) for juvenile and yearling river otter males collected from Oregon and Washington, 1994-99.

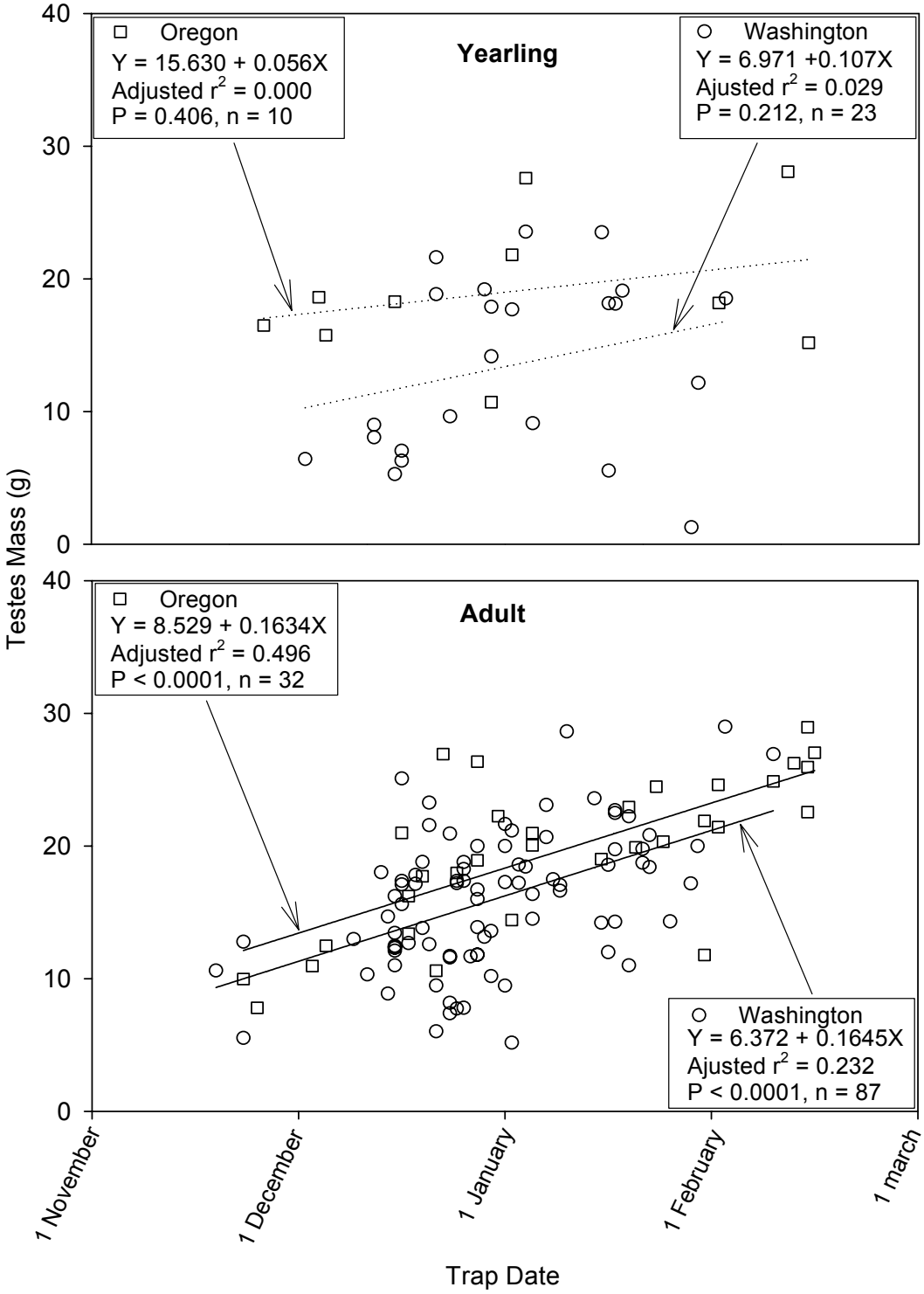


Figure 16. Relationships between testes mass (g) and trap date (by state) for yearling and adult river otter males collected from Oregon and Washington, 1994-99.

Table 7. Mean baculum mass and length in relation to age for river otter males collected from Oregon and Washington, 1994-99.

Age (years)	n	Mass (g)		Length (mm)	
		Mean ± SE[a]	Range	Mean ± SE[a]	Range
<1	61	2.62 ± 0.09 C	1.09 - 4.54	81.6 ± 0.77 B	61.7 - 95.1
1	46	5.98 ± 0.19 B	3.07 - 9.64	95.5 ± 0.79 A	79.9 - 105
2	44	6.28 ± 0.19 AB	2.78 - 8.79	96.2 ± 0.78 A	84.5 - 106
3	26	7.08 ± 0.25 AB	4.59 - 9.32	99.4 ± 0.99 A	87.0 - 108
4	21	6.95 ± 0.28 AB	5.17 - 9.25	98.4 ± 1.54 A	83.6 - 113
5	13	7.14 ± 0.20 AB	5.68 - 8.21	99.2 ± 1.40 A	89.1 - 107
6	11	7.44 ± 0.46 A	5.59 - 10.5	99.8 ± 1.46 A	94.8 - 108
7	7	6.97 ± 0.20 AB	6.27 - 7.58	99.0 ± 1.25 A	93.2 - 104
8	11	7.28 ± 0.45 AB	4.75 - 9.69	98.5 ± 1.83 A	90.4 - 112
>8	9	7.13 ± 0.35 AB	5.92 - 8.69	96.0 ± 0.97 A	91.7 - 99.4

[a] One way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Column means sharing the same letters are not significantly different from each other. SE = standard error. P for each column tested was < 0.0001.

otter femurs and humeri reach their maximum lengths after the age of one (Figure 17). Maximum baculum mass appears also to occur after the age of three, while femur, humerus and scapula mass increases until after six years of age (Figure 18).

Ten river otters were not skinned when received and a comparison was made between the body weights of un-pelted versus pelted carcasses. The equation in Figure 19 (with the adjusted r^2 of 0.975) can be used to effectively estimate an otter's original body mass prior to pelt removal.

Hormone Analyses

The HPLC method used gave good baseline separation for the standard

Table 8. Mean femur mass and length, humerus mass and length and scapula mass in relation to age for river otter males collected from Oregon and Washington, 1996-99.

	Femur			Humerus			Scapula	
Age		Mass (g)[a]	Length (mm)[a]		Mass (g)[a]	Length (mm)[a]		Mass (g)[a]
(years)	n	Mean ± SE	Mean ± SE	n	Mean ± SE	Mean ± SE	n	Mean ± SE
<1	58	9.00 - 0.20 B	77.7 - 0.55 B	56	9.65 - 0.22 C	77.1 - 0.58 B	56	4.48 - 0.11 B
1	38	11.0 - 0.20 A	83.0 - 0.62 A	37	12.0 - 0.21 - AB	81.9 - 0.63 AB	38	6.04 - 0.13 A
2	41	10.9 - 0.23 A	82.3 - 0.56 A	39	11.9 - 0.28 B	81.3 - 0.59 AB	40	5.87 - 0.14 A
3	23	11.4 - 0.26 A	82.6 - 0.56 A	22	12.4 - 0.32 AB	81.7 - 0.72 AB	23	6.17 - 0.14 A
4	18	11.5 - 0.40 A	82.9 - 0.97 A	18	13.0 - 0.40 AB	81.5 - 1.11 AB	18	6.32 - 0.24 A
5	12	12.0 - 0.47 A	82.7 - 1.00 A	12	13.3 - 0.48 AB	80.5 - 1.07 AB	12	6.55 - 0.26 A
6	11	12.0 - 0.40 A	84.9 - 0.93 A	11	13.4 - 0.47 AB	83.6 - 1.12 A	10	6.82 - 0.23 A
7	7	11.6 - 0.62 A	80.6 - 2.17 AB	7	12.6 - 0.58 AB	79.8 - 2.29 AB	7	6.10 - 0.16 A
8	9	12.3 - 0.46 A	82.4 - 0.96 A	9	13.3 - 0.49 AB	81.3 - 1.05 AB	10	6.82 - 0.32 A
>8	8	12.5 - 0.49 A	84.5 - 0.83 A	8	13.8 - 0.47 A	82.5 - 0.81 A	8	6.77 - 0.30 A

[a] One way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Column means sharing the same letters are not significantly different from each other. SE = standard error. P for each column tested was <0.0001.

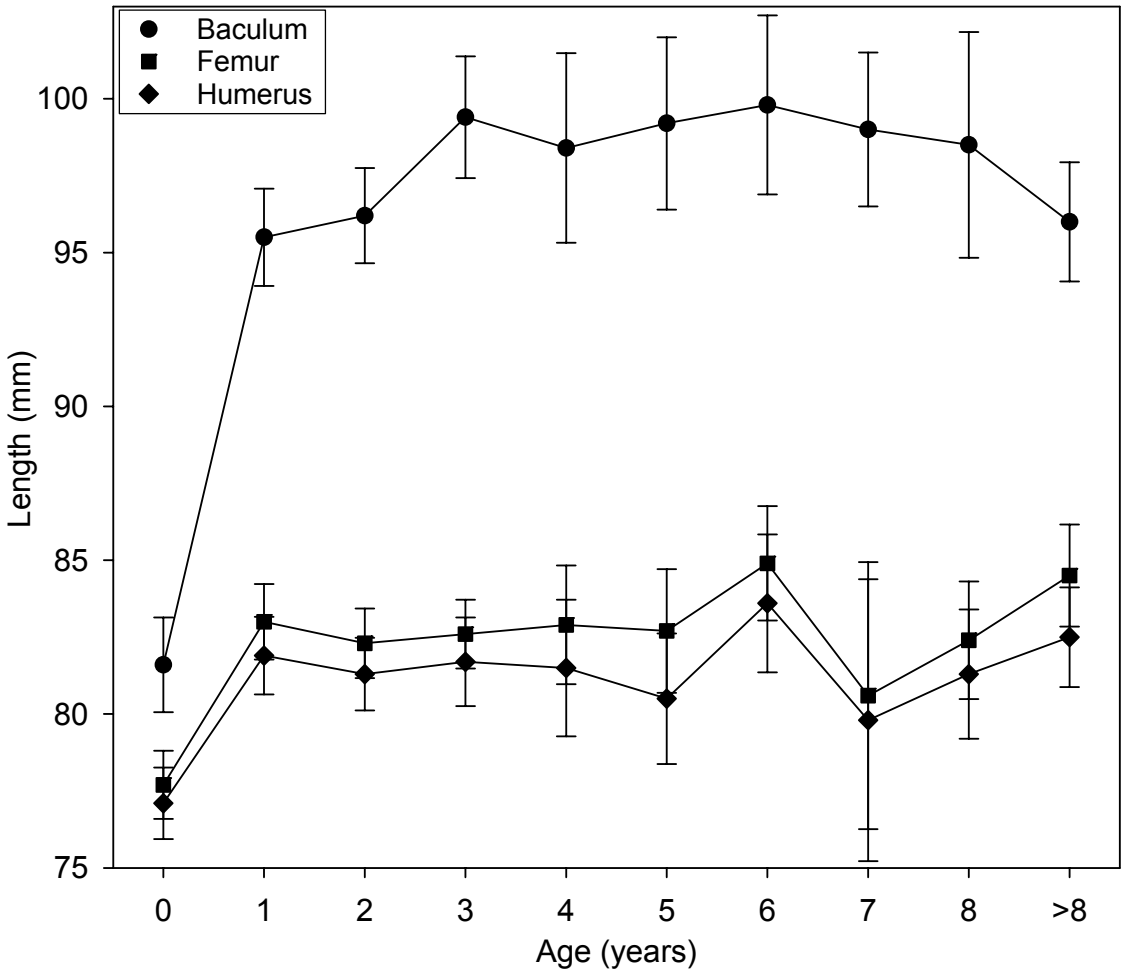


Figure 17. Mean baculum, femur and humerus length (mm) by age in years for river otter males collected from Oregon and Washington, 1994-99. Error bars represent plus or minus 2 SE of the mean.

peaks of androstenedione, testosterone, hydroxyprogesterone, and progesterone (Figure 20). Standard extraction recoveries (n = 10) were 100% (sd = 6.3) for androstenedione, 99% (sd = 6.8) for testosterone, 103% (sd = 8.1) for hydroxyprogesterone and 87% (sd = 5.2) for progesterone. Method extraction and cleanup worked well for bull testis as illustrated in Figure 21. Recoveries of bull testis extraction spikes (n =13) were lower, with 98.6% (sd = 4.8) for androstenedione, 84.7% (sd = 7.3) for hydroxyprogesterone and 57.6%

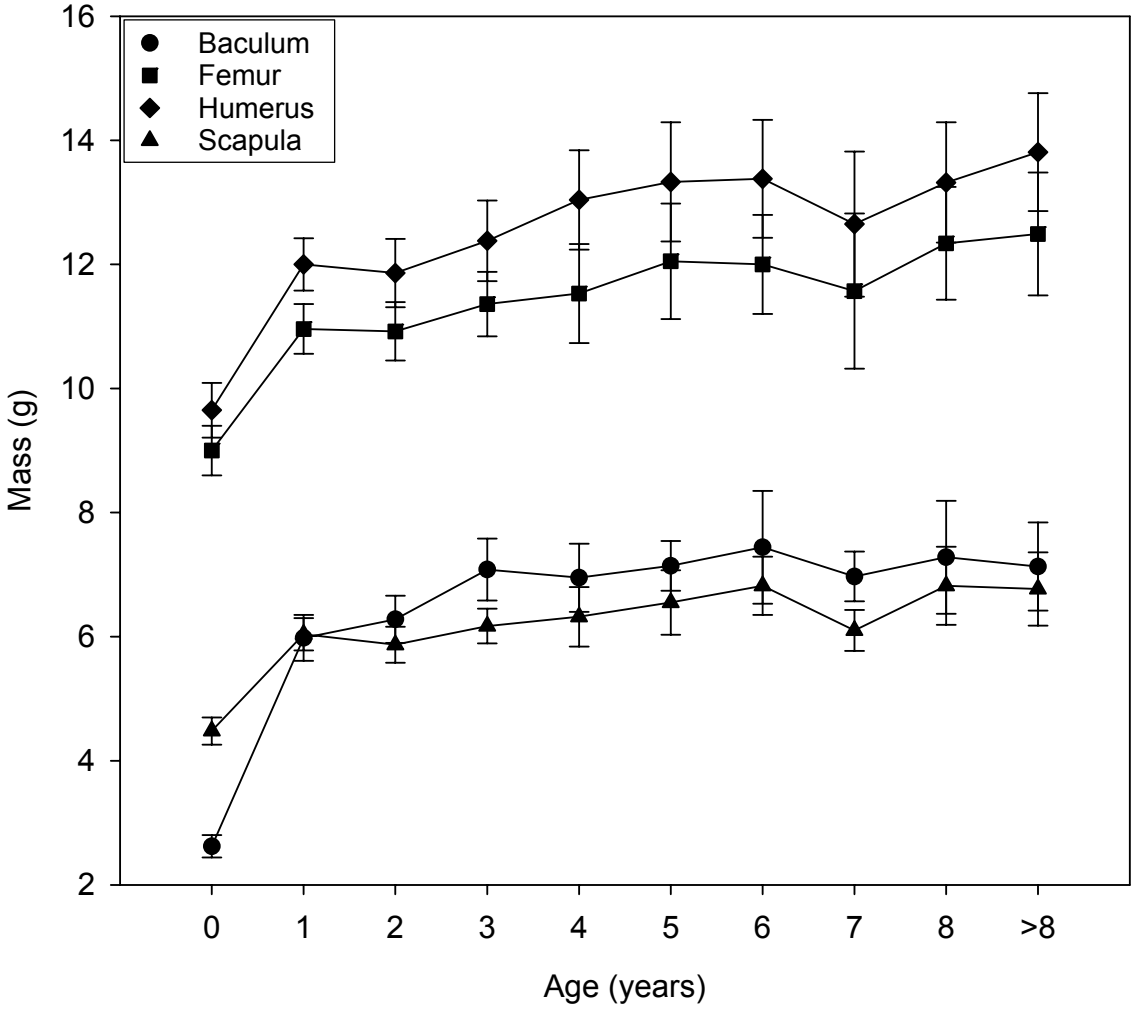


Figure 18. Mean baculum, femur, humerus and scapula mass (g) by age in years for river otter males collected from Oregon and Washington, 1994-99. Error bars represent plus or minus 2 SE of the mean.

(sd = 6.9) for progesterone. Mean testosterone for bull testis tissue used was 1632 ng/g (n = 19, sd = 154) which is within the normal range of testicular testosterone concentrations for bulls. Testosterone chromatograms are shown in Figures 22 and 23 for a juvenile male (RAG-040) and a yearling male (RAG99-06), respectively.

Testicular testosterone concentrations were analyzed for in 77 male river otters (11 juveniles, 11 yearlings, 55 adults). Testosterone levels of juvenile male otters were all below the lowest level of quantitation. Yearling males showed a significant increase in testosterone beginning about 1 January and peaking about 1 March (Figure 24). Adult males showed a pattern of testosterone increase similar to the yearlings males (Figure 24).

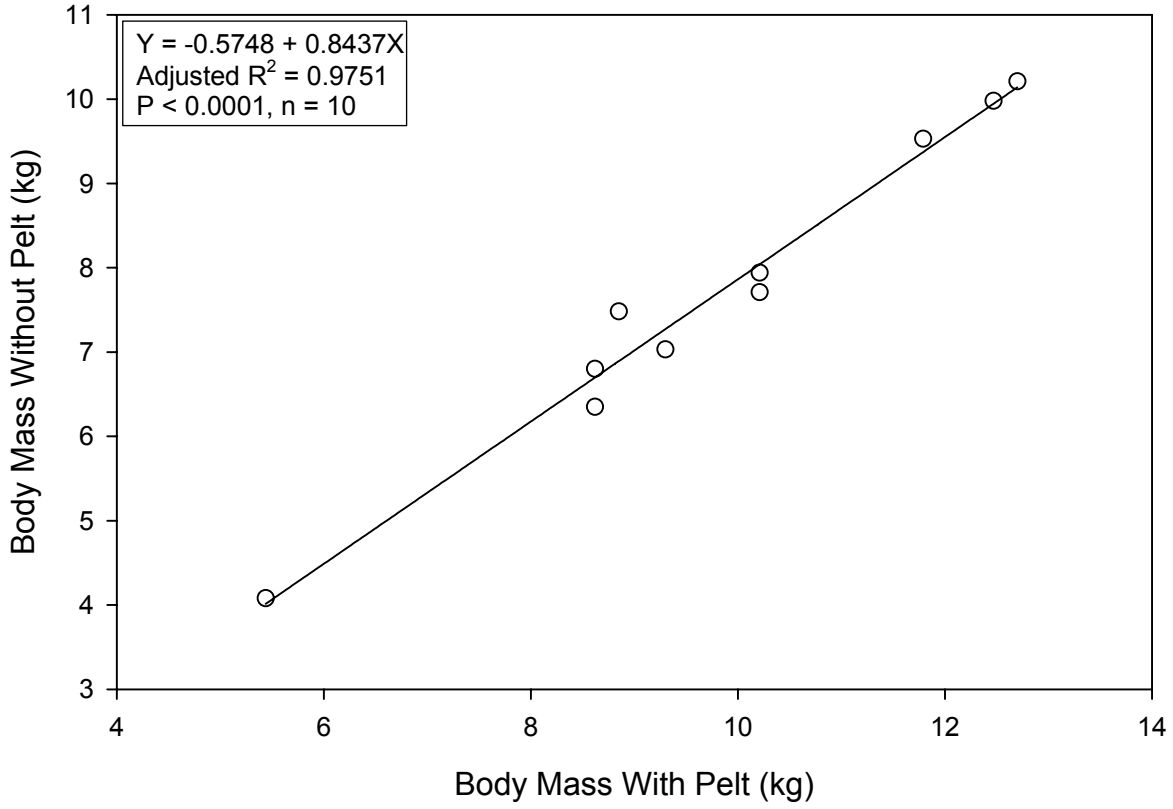


Figure 19. Relationship between body mass (kg) with and without pelts from unskinned river otter carcasses collected from Oregon and Washington, 1996-99. Regression equation used to estimate the unskinned body mass of pelted otters received from trappers.

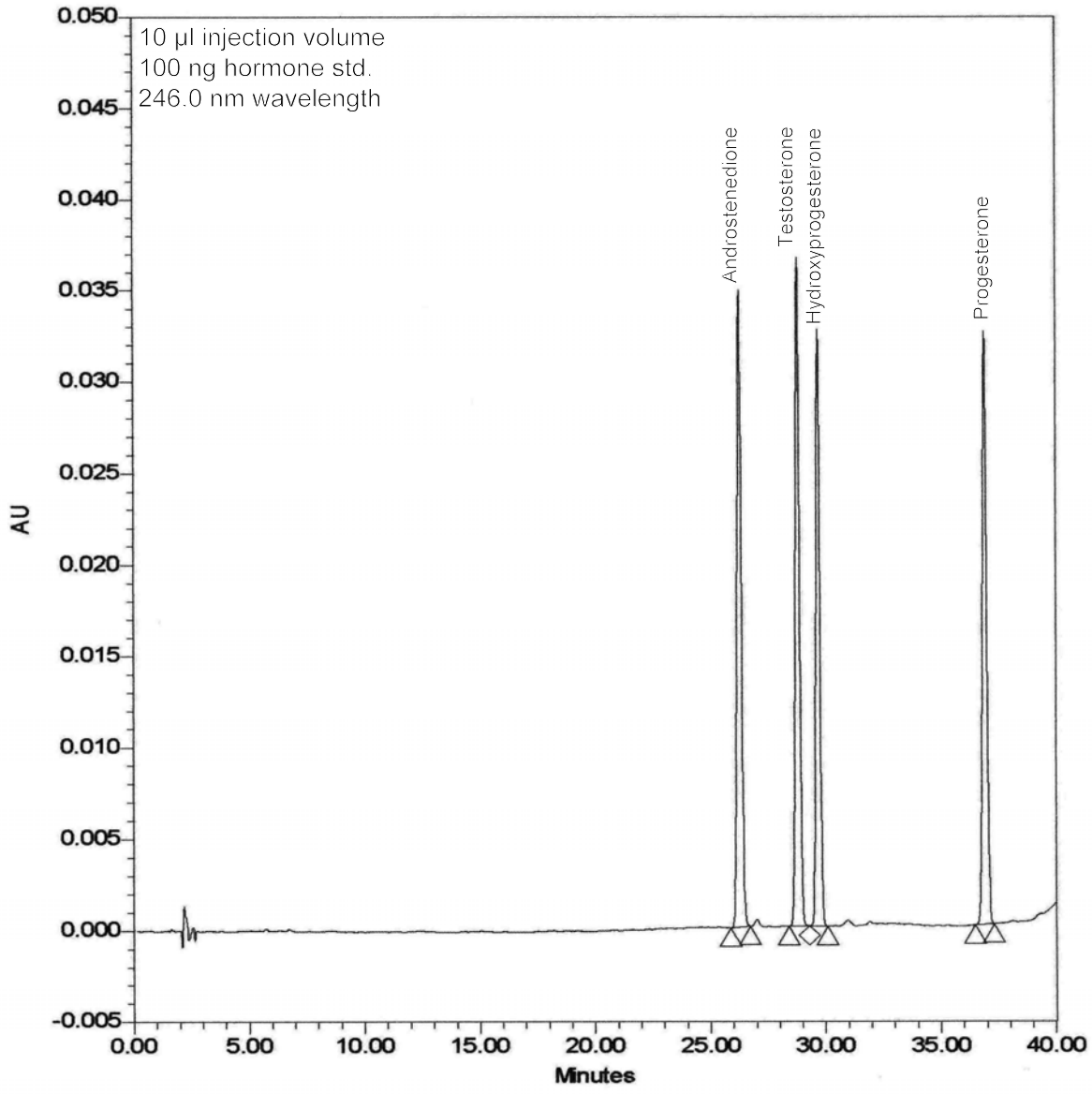


Figure 20. Chromatogram of steroid hormone standard mixture, showing good baseline separation between peaks.

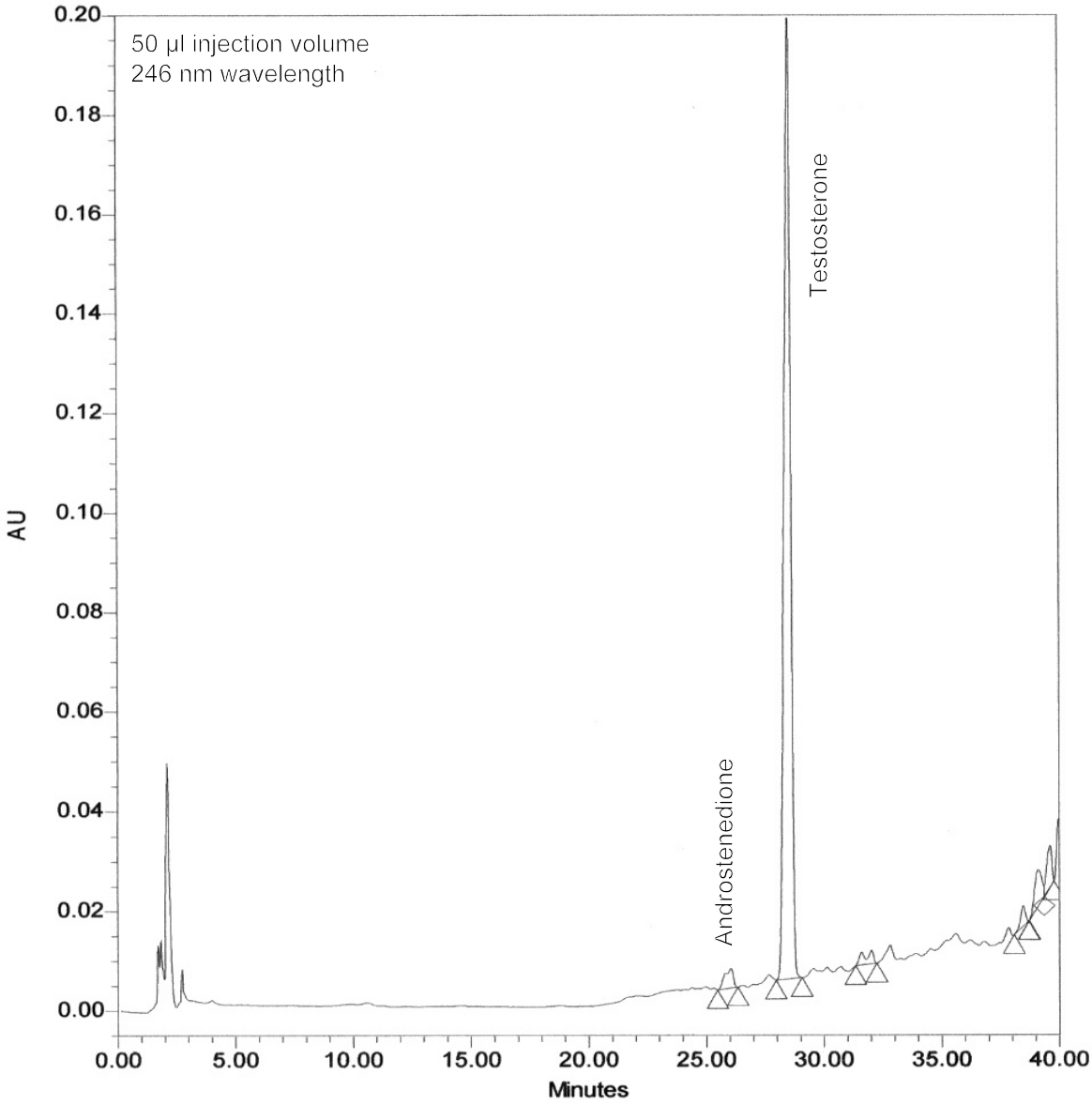


Figure 21. Chromatogram of bovine testicular steroid hormone extract. Testosterone concentration at 1835.9 ng g^{-1}, ww.

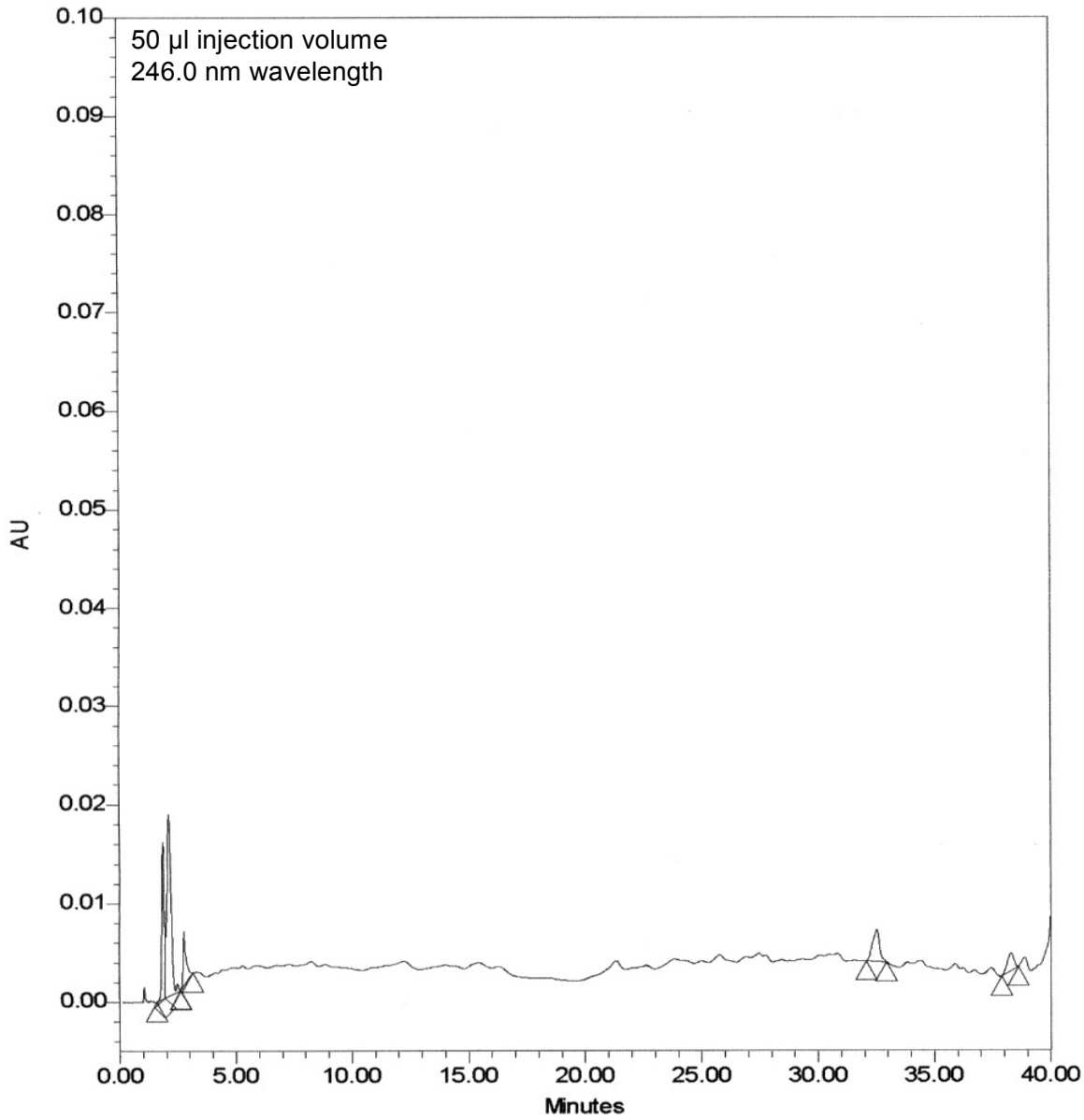


Figure 22. Chromatogram of testicular steroid hormone extract from Juvenile river otter male RAG-040. No testosterone detected.

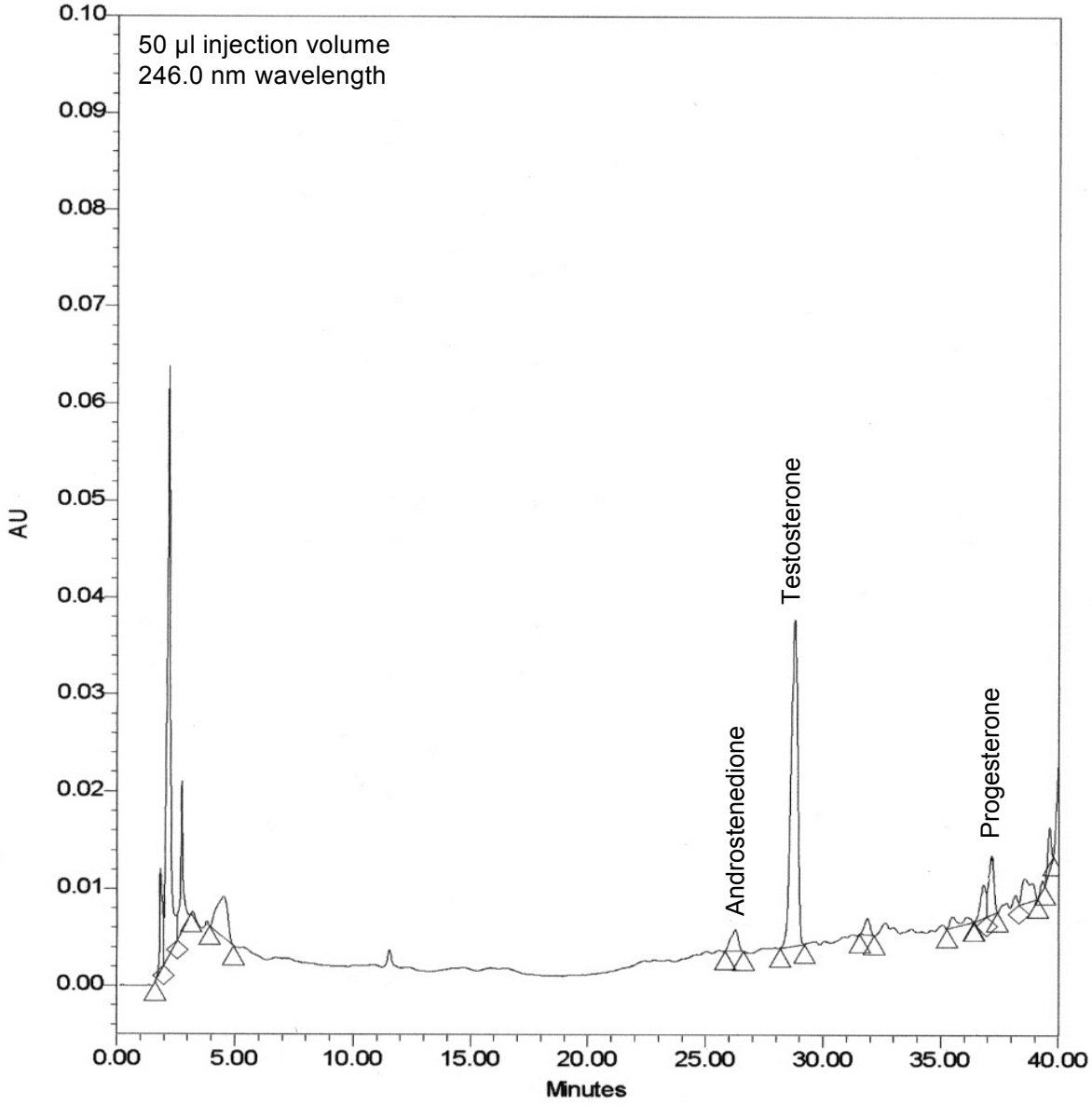


Figure 23. Chromatogram of testicular steroid hormone extract from yearling river otter male RAG99-06. Testosterone concentration at 237.2 ng g^{-1}.

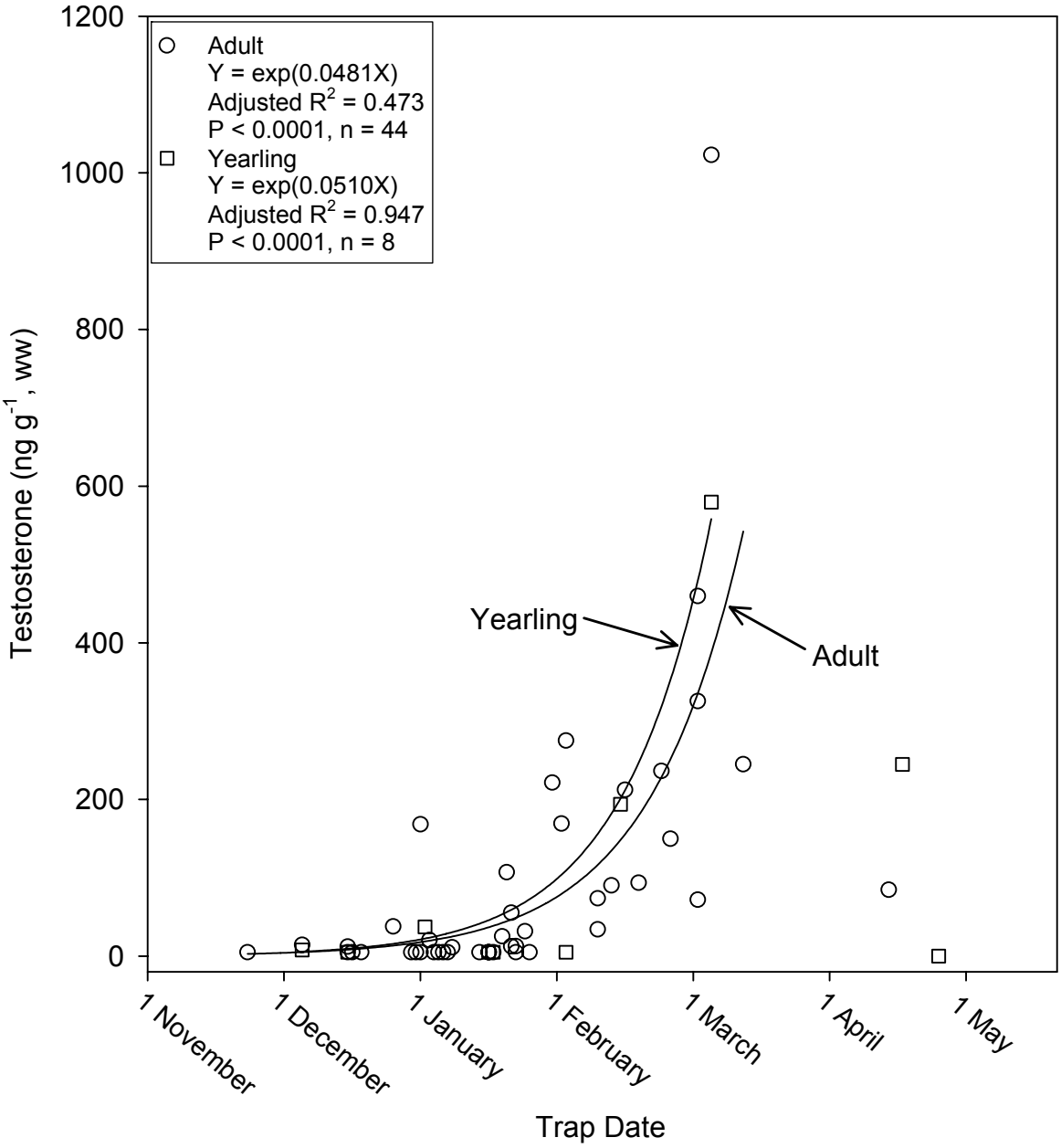


Figure 24. Relationship between trap date and testes testosterone concentrations in adult and yearling river otter males collected from Oregon and Washington, 1996-99. The curves are calculated excluding the 3 data points after 1 April.

Contaminant Chemistry

Contaminants were analyzed in the livers of 196 river otters collected from Oregon and Washington (1 juvenile female, 63 juvenile males, 6 yearling females, 21 yearling males, 9 adult females, and 96 adult males). Contaminant residues of females and yearlings were not used in the statistical analyses. This study concentrated on contaminant effects with males only. Yearling males were not used in this analysis because of their long-range dispersal and wandering. This age class can move considerable distances after reaching 7 to 12 months of age, with some dispersing yearlings known to travel up to 200 km (125 miles) from their natal areas in Idaho (Hornocker et al. 1983, Melquist and Hornocker 1983). Only residues of adult and juvenile males were used, because of the confounding potential of yearling movements. Again, metals are reported on a dry weight basis while organic contaminants are reported on a wet weight basis.

Correlations between metals analyzed (Appendix 1) showed significant correlations for those metals that are physiologically required (i.e., Ca, Cu, Fe, K, Mg, Mn, Na, Zn). Cadmium was significantly correlated with Fe and Hg, with Hg also correlated with Ca, Mn and Zn (Appendix 1). Cadmium and Hg also had significant correlations with several OCs, PCBs, PCDDs and PCDFs, though all correlations were negative (Appendix 2). The OCs and their metabolites were highly correlated with each other except for photo-mirex and β-HCH (Appendix 3). The OCs and metabolites were also highly correlated with the PCBs except for PCBs 87, 105, 77, 81 and 126. Several of the higher chlorinated PCDDs and PCDFs (hexa-, hepta- and octa-) were correlated with the OCs and their metabolites, but not as strongly. The PCBs were all strongly correlated with each other except for PCBs 105, 77 and 81 (Appendix 3). Polychlorinated biphenyls were moderately correlated with PCDDs and PCDFs, with stronger

correlations associated with the higher chlorinated PCBs (i.e., hexa-, hepta-, octa- and nona-). The PCDDs and PCDFs were strongly correlated with each other, again for the higher chlorinated compounds (Appendix 3).

No significant associations were found between metals of concern (i.e., Cd and Hg) and age for adult males (see Figure 25 for relationship between Hg and age). Also, no relationships were found for OCs, PCBs, PCDDs and PCDFs (e.g., see Figure 26 and 27 for DDE and ΣPCBs).

The percent moisture and lipid in livers were not significantly different between regional locations for either juvenile or adult male otters (Tables 9 and 10). The mean percent moisture in livers of juvenile males (71.5, n = 60) and adult males (70.8, n = 94) was similar, but significantly different (P = 0.0389) (Table 11). The mean liver lipid (3.41%) was higher for adult males than juvenile males (2.60%)(P = 0.0002).

Metals

Hepatic concentrations of the metals Al, As, Cd, Cr, Co, Pb, Ni, and V were all below the LOQ for the juvenile male otters, with Cr, Co, Pb, Ni, and V below the LOQ for adult males (Tables 9 and 10). Aluminum was quantified in the livers of two adult males from the OP, an 8 year-old male (RAG-144) trapped along the Soleduck River with 19.2 μg g^{-1} and an 8 year-old male (RAG-221) trapped along Petroleum Creek north of Ozzette Lake with 14.8 μg g^{-1}. Arsenic was not analyzed in otters trapped from the LCR in 1994. Arsenic was quantified in the liver of one juvenile male from SW (RAG-204, 5.51 μg g^{-1}) and 22 of 87 adult males from five of the seven regional locations, with concentrations ranging from nd to 8.03 μg g^{-1}. A geometric mean for As was calculated only for SW (Table 10), because at least half of the samples from the other locations were below the detection limit.

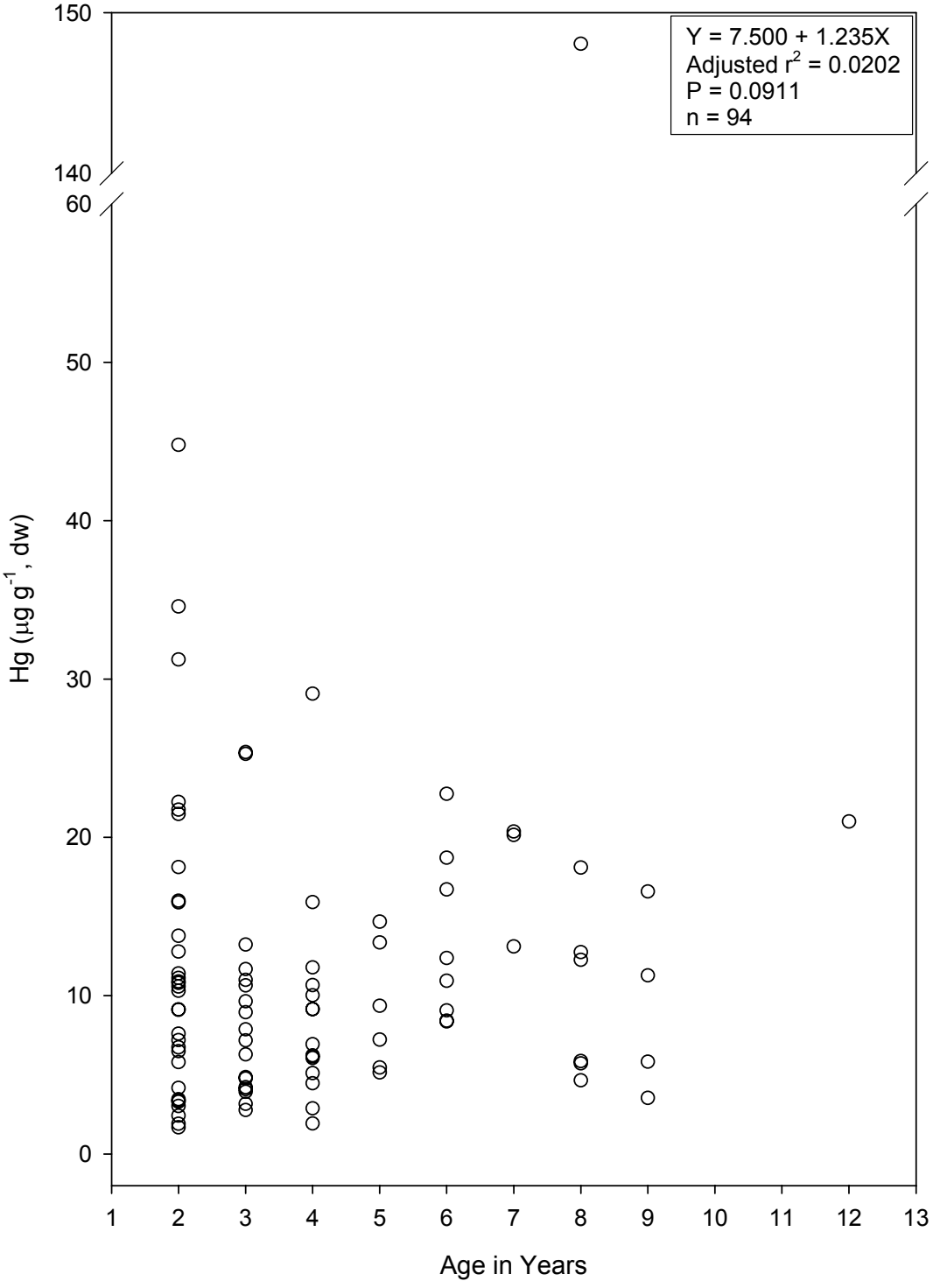


Figure 25. Relationship between hepatic Hg and age of adult river otter males collected from Oregon and Washington, 1994-99.

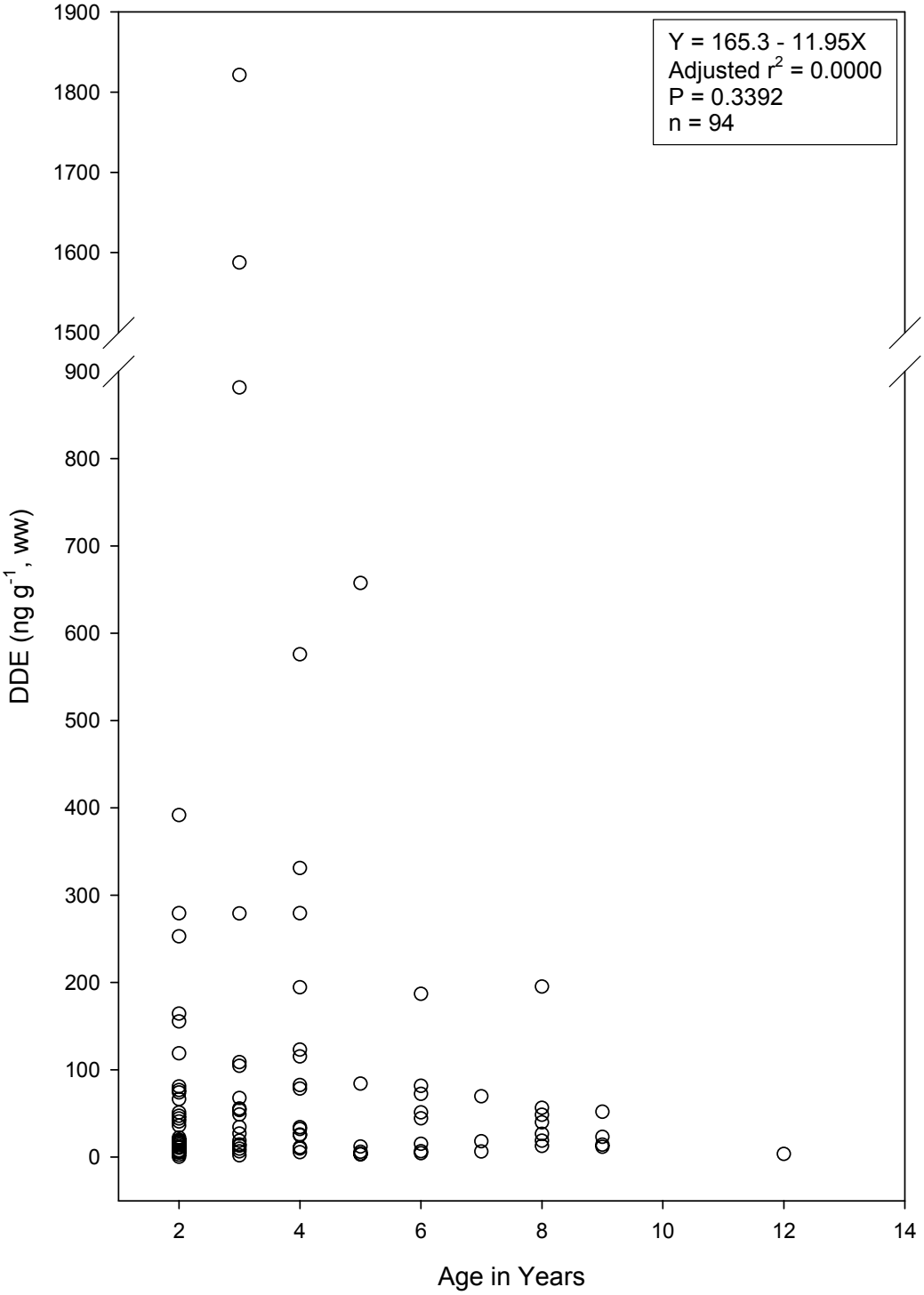


Figure 26. Relationship between hepatic DDE and age of adult river otter males collected from Oregon and Washington, 1994-99.

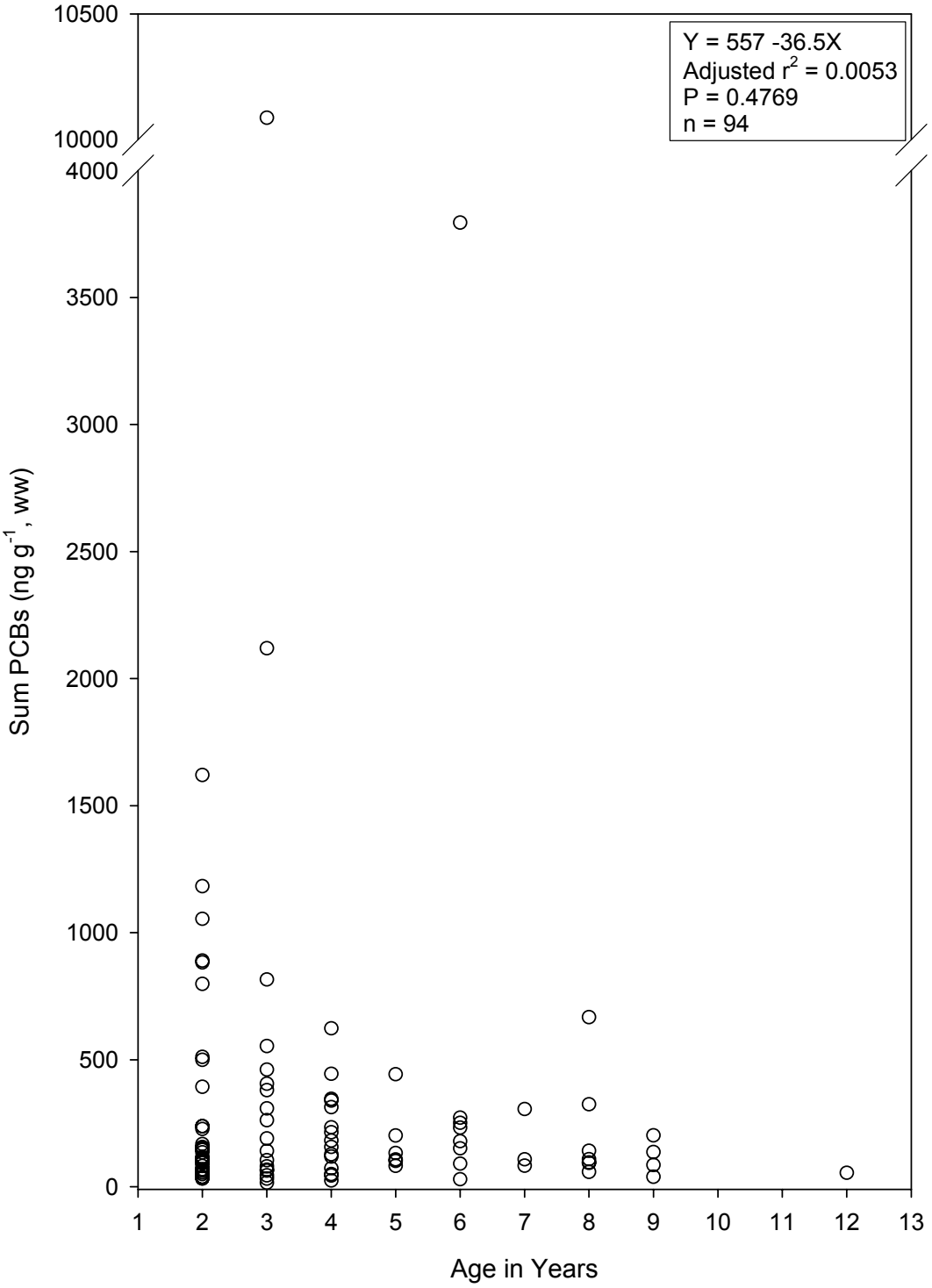


Figure 27. Relationship between hepatic sum of PCBs analyzed and age of adult river otter males collected from Oregon and Washington, 1994-99.

Table 9. Regional location comparisons using geometric mean metal (μg g^{-1}, dw) and organochlorine insecticide and metabolite concentrations (μg kg^{-1}, ww) in livers of juvenile river otter males collected in Oregon and Washington, 1994-99. Percent moisture and lipid content of livers are arithmetic means. LCR = Lower Columbia River, OC = Oregon Coast, OP = Olympic Peninsula, PS = Puget Sound, SW = Southwestern Washington, WB = Willamette River Basin, and WW = Western Washington.

Location	LCR	OC	OP	PS	SW	WB	WW
n	6	7	7	12	14	8	6
% Moisture	70.5 B	72.7 A	71.0 B	71.7 AB	71.3 AB	72.0 AB	71.0 B
% lipid	3.26 A	2.35 A	2.38 A	2.42 A	2.73 A	2.37 A	2.78 A
Aluminum	6 nd	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
Arsenic	6 na	8 nd	7 nd	12 nd	13 nd	8 nd	6 nd
Cadmium	6 nd	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
Chromium	6 nd	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
Cobalt	6 nd	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
Copper	31.4 A	44.2 A	38.3 A	31.4 A	36.1 A	27.9 A	25.9 A
Iron	745 A	733 A	1040 A	750 A	871 A	787 A	989 A
Lead	6 nd	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
Manganese	**6.81 B**	**9.77 AB**	**9.81 AB**	**10.2 A**	**10.7 A**	**11.1 A**	**9.10 AB**
Mercury	3.63 A	5.01 A	8.05 A	3.74 A	7.84 A	5.55 A	5.85 A
Nickel	5 nd	7 nd	7 nd	12 nd	12 nd	7 nd	6 nd
Vanadium	6 nd	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
Zinc	**70.6 B**	**106 A**	**95.5 AB**	**91.1 AB**	**91.2 AB**	**84.8 AB**	**73.4 AB**
1,2,3,4 - TCB	6 nd	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
1,2,4,5 - TCB	6 nd	8 nd	7 nd	12 nd	13 nd	8 nd	6 nd
QCB	4 nd	7 nd	7 nd	0.04	11 nd	6 nd	5 nd
HCB	3.21 A	3.27 A	5.02 A	4.29 A	6.90 A	6.45 A	3.37 A
OCS	0.17	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
DDE	51.2 A	9.58 A	11.8 A	18.9 A	18.7 A	57.1 A	11.8 A
DDD	3.56 A	0.20 A	0.37 A	1.09 A	1.12 A	2.11 A	0.51 A

Table 9. Continued.

Location	LCR	OC	OP	PS	SW	WB	WW
n	6	7	7	12	14	8	6
DDT	0.03	8 nd	7 nd	9 nd	13 nd	8 nd	5 nd
Mirex	**0.62 A**	7 nd	**0.03 B**	**0.05 AB**	**0.07 AB**	8 nd	**0.08 AB**
Photomirex	4 nd	7 nd	6 nd	12 nd	12 nd	8 nd	6 nd
α- HCH	6 nd	7 nd	6 nd	7 nd	13 nd	7 nd	6 nd
β - HCH	0.14 A	0.03 A	4 nd	0.27 A	0.06 A	0.07 A	0.01 A
γ - HCH	6 nd	8 nd	6 nd	12 nd	14 nd	8 nd	6 nd
Oxychlordane	**5.28 AB**	**3.74 AB**	**2.71 B**	**4.74 AB**	**4.91 AB**	**11.1 A**	**3.83 AB**
trans-Chlordane	4 nd	8 nd	6 nd	12 nd	13 nd	8 nd	5 nd
cis-Chlordane	0.14 A	8 nd	6 nd	10 nd	10 nd	5 nd	0.03 A
trans-Nonachlor	2.33 A	0.60 A	1.87 A	1.37 A	1.81 A	4.73 A	1.29 A
cis-Nonachlor	0.31 A	0.04 A	0.05 A	0.20 A	0.08 A	0.26 A	0.13 A
Heptachlor Epoxide	0.90 A	0.40 A	0.36 A	0.81 A	0.55 A	0.93 A	0.22 A
Dieldrin	**3.91 AB**	**1.09 BC**	**1.20 BC**	**2.98 ABC**	**2.08 ABC**	**7.40 A**	**0.87 C**

One-way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows of the categories sharing the same letters are not significantly different. # nd = number of samples with non detections. na = not analyzed. TCB = tetrachlorobenzene, QCB = pentachlorobenzene, HCB = hexachlorobenzene, OCS = octachlorostyrene, DDE = dichlorodiphenyldichloroethylene, HCH = hexachlorocyclohexane, DDD = dichlorodiphenyldichloroethane, DDT = p,p'-dichlorodiphenyltrichloroethane.

Table 10. Regional location comparisons using geometric mean metal (µg g^{-1}, dw) and organochlorine insecticide and metabolite concentrations (µg kg^{-1}, ww) in livers of adult river otter males collected in Oregon and Washington, 1994-99. Percent moisture and lipid content of livers are arithmetic means. LCR = Lower Columbia River, OC = Oregon Coast, OP = Olympic Peninsula, PS = Puget Sound, SW = Southwestern Washington, WB = Willamette River Basin, and WW = Western Washington.

Location	LCR	OC	OP	PS	SW	WB	WW
n	9	17	18	12	16	17	5
% Moisture	69.9 A	70.7 A	70.3 A	71.3 A	70.6 A	71.5 A	72.1 A
% Lipid	3.83 A	3.39 A	3.97 A	2.68 A	3.46 A	2.81 A	4.09 A
Aluminum	9 nd	17 nd	16 nd	12 nd	16 nd	17 nd	5 nd
Arsenic	9 na	12 nd	12 nd	11 nd	3.85	14 nd	5 nd
Cadmium	8 nd	0.42 A	0.47 A	0.42 A	0.55 A	10 nd	4 nd
Chromium	9 nd	17 nd	18 nd	12 nd	16 nd	17 nd	5 nd
Cobalt	9 nd	17 nd	18 nd	12 nd	16 nd	17 nd	5 nd
Copper	25.1 A	20.0 A	32.4 A	25.0 A	26.0 A	26.0 A	33.2nd A
Iron	1025 A	982 A	1066 A	885 A	1218 A	1164 A	982 A
Lead	9 nd	17 nd	18 nd	12 nd	16 nd	17 nd	5 nd
Manganese	**6.54 B**	**7.71 AB**	**9.00 A**	**8.91 A**	**7.64 AB**	**7.77 AB**	**9.55 A**
Mercury	**3.46 B**	**9.23 A**	**12.6 A**	**7.89 AB**	**10.0 A**	**9.26 A**	**13.8 A**
Nickel	9 nd	16 nd	18 nd	12 nd	16 nd	17 nd	5 nd
Vanadium	9 nd	17 nd	18 nd	12 nd	16 nd	17 nd	5 nd
Zinc	**61.0 C**	**70.2 BC**	**83.1 AB**	**92.6 A**	**74.0 ABC**	**73.3 ABC**	**76.6 ABC**
1,2,3,4 - TCB	9 nd	17 nd	18 nd	12 nd	16 nd	17 nd	5 nd
1,2,4,5 - TCB	9 nd	15 nd	18 nd	12 nd	14 nd	16 nd	5 nd
QCB	7 nd	12 nd	**0.03 B**	7 nd	**0.04 AB**	**0.05 AB**	**0.24 A**
HCB	6.27 A	5.39 A	8.59 A	5.17 A	6.61 A	8.00 A	7.24 A
OCS	0.57	14 nd	18 nd	12 nd	16 nd	17 nd	5 nd
DDE	**143 A**	**16.0 B**	**31.2 AB**	**20.2 B**	**18.8 B**	**85.9 AB**	**78.0 AB**
DDD	**10.3 A**	**0.46 B**	**1.67 AB**	**1.31 AB**	**0.93 AB**	**7.38 A**	**5.95 AB**

Table 10. Continued.

Location	LCR	OC	OP	PS	SW	WB	WW
n	9	17	18	12	16	17	5
DDT	0.07 A	13 nd	0.09 A	9 nd	9 nd	11 nd	0.15 A
Mirex	**1.23 A**	**0.12 BC**	**0.32 ABC**	**0.06 C**	**0.17 ABC**	9 nd	**0.67 AB**
Photomirex	7 nd	10 nd	0.27 A	12 nd	0.13 A	17 nd	5 nd
α-HCH	7 nd	16 nd	18 nd	7 nd	16 nd	17 nd	5 nd
β-HCH	**0.06 B**	**0.07 B**	**0.15 AB**	**0.74 A**	**0.11 AB**	11 nd	**0.02 B**
γ-HCH	9 nd	17 nd	18 nd	12 nd	16 nd	16 nd	5 nd
Oxychlordane	12.9 A	5.92 A	7.33 A	9.74 A	5.28 A	14.5 A	13.5 A
trans-Chlordane	0.09	14 nd	18 nd	9 nd	14 nd	11 nd	4 nd
cis-Chlordane	0.40 A	13 nd	12 nd	0.04 A	14 nd	0.13 A	0.12 A
trans-Nonachlor	7.15 A	1.25 A	3.27 A	3.16 A	1.41 A	6.94 A	5.96 A
cis-Nonachlor	**0.88 A**	**0.04 B**	**0.15 AB**	**0.32 AB**	**0.09 AB**	**0.38 AB**	**0.64 A**
Heptachlor Epoxide	**1.30 AB**	**0.32 C**	**0.56 ABC**	**1.22 AB**	**0.46 BC**	**1.82 A**	**1.23 AB**
Dieldrin	**6.82 AB**	**1.47 C**	**1.96 BC**	**4.58 ABC**	**1.79 BC**	**13.9 A**	**6.28 AB**

One-way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows of the categories sharing the same letters are not significantly different. # nd = number of samples with non detections. na = not analyzed. TCB = tetrachlorobenzene, QCB = pentachlorobenzene, HCB = hexachlorobenzene, OCS = octachlorostyrene, DDE = p,p'-dichlorodiphenyldichloroethylene, HCH = hexachlorocyclohexane, DDD = p,p'-dichlorodiphenyldichloroethane, DDT = p,p'-dichlorodiphenyltrichloroethane.

Table 11. Age comparisons using geometric mean organochlorine insecticide and metabolite concentrations (µg kg^{-1}, ww) in livers of juvenile and adult river otter males collected in Oregon and Washington, 1994-99.

	Juvenile (n = 60)			Adult (n = 94)		
Chemical	Mean	Range	sd	Mean	Range	sd
% Moisture	**71.5 A**	69.1 - 74.5	1.20	**70.8 B**	58.5 - 83.1	2.19
% Lipid	**2.60 B**	1.45 - 5.17	0.69	**3.41 A**	1.23 - 13.1	1.55
Copper	**33.4 A**	11.2 - 102	0.21	**26.0 B**	12.1 - 71.5	0.19
Iron	**832 B**	351 - 1370	0.14	**1058 A**	394 - 2216	0.11
Manganese	**9.80 A**	4.30 - 18.2	0.12	**8.10 B**	4.64 - 14.5	0.11
Mercury	**5.53 B**	1.35 - 22.5	0.27	**9.06 A**	1.68 - 148	0.09
Zinc	**88.1 A**	51.7 - 171	0.12	**75.7 B**	54.6 - 165	0.33
HCB	**4.91 B**	0.93 - 74.8	0.35	**6.72 A**	0.78 - 572	0.37
DDE	**20.7 B**	0.46 - 1036	0.59	**35.0 A**	0.33 - 1821	0.66
DDD	**0.93 B**	6 nd - 45.6	0.90	**1.93 A**	6 nd - 1443	0.98
Mirex	**0.05 B**	26 nd - 1.35	0.94	**0.16 A**	16 nd - 4.37	0.92
β - HCH	0.06 A	26 nd - 3.56	1.05	0.08 A	16 nd - 2.68	0.93
Oxychlordane	**5.01 B**	0.19 - 17.9	0.39	**8.53 A**	0.99 - 138	0.38
trans-Nonachlor	**1.69 B**	2 nd - 28.0	0.68	**3.02 A**	1 nd - 282	0.66
cis-Nonachlor	0.12 A	12 nd - 2.82	0.80	0.18 A	18 nd - 77.8	0.92
Heptachlor Epoxide	0.58 A	2 nd - 4.83	0.54	0.76 A	1 nd - 23.9	0.48
Dieldrin	**2.29 B**	0.12 - 52.3	0.49	**3.49 A**	0.25 - 267	0.57

One-way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows sharing the same letters are not significantly different.
nd = number of samples with non-detections. sd = standard deviation.

Cadmium was found in the livers of 46 of 96 adult otters from all locations, but not in juveniles. No significant difference in CD was found between locations where geometric means could be calculated (Table 10). Cadmium concentrations ranged from nd to 1.18 µg g^{-1}. Cadmium was most frequently reported at four regional locations (OC, OP, PS, and SW). The highest individual Cd concentration was from an 8 year-old male (RAG-144) from the Olympic Peninsula at 1.18 µg g^{-1}. Copper and Fe were found in all livers analyzed, but concentrations were not significantly different among regional locations for either juvenile or adult male otters (Tables 9 and 10). Overall, Cu concentrations were higher in juvenile males (33.4 µg g^{-1}) than adult males (26.0

μg g^{-1})(P = 0.001)(Table 11), while Fe was higher in adults (1058 μg g^{-1}) than juveniles (832 μg g^{-1})(P < 0.0001). Manganese was lowest for the LCR when compared to other locations for both adult and juvenile male otters. Juveniles (9.80 μg g^{-1}) had a higher mean Mn than did adults (8.10 μg g^{-1})(P < 0.0001).

Regionally, geometric mean Hg was also lowest for the LCR for both age groups, but only significant for adult males. Overall, geometric mean Hg was significantly higher for adult males (9.06 μg g^{-1}) than juveniles (5.53 μg g^{-1}) (P < 0.0001). The highest individual adult and juvenile Hg concentrations were found with otters RAG-221 (adult, 148 μg g^{-1}) and RAG-201 (juvenile, 22.45 μg g^{-1}) from Petroleum Creek on the Olympic Peninsula. A total of 46 male otters of both age groups contained above 10 μg g^{-1} (30%), with 15 (9.7%) above 20 μg g^{-1}. Only 1 male adult (RAG98-02, 11.9 μg g^{-1} Hg) collected east of the Cascade Mountains on the Metolius River of Oregon had a liver Hg concentration above 10 μg g^{-1}. Finally, geometric mean Zn was significantly higher for juvenile males from OC than juveniles from LCR (Table 9). Adult males from PS were higher in Zn than adult males from OC and LCR (Table 10). Mean Zn was higher in juvenile males (88.1 μg g^{-1}) than adult males (75.7 μg g^{-1})(P = 0.0002)(Table 11).

OC Insecticides and Metabolites

No statistical comparisons could be made between juvenile male regional location means for the following OCs 1,2,3,4-tetrachlorobenzene (1,2,3,4-TCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TCB), pentachlorobenzene (QCB), octachlorostyrene (OCS), DDT, photomirex, α-hexachlorohexane (α-HCH), γ-hexachlorohexane (γ- HCH) and trans-chlordane because >50% of the samples were non-detections for each contaminant and geomatric means were not calculated (Table 9). The same was also true for adult male otters, except for QCB, DDT and photomirex (Table 10). No significant differences in geometric mean were found between locations for juvenile male hepatic concentrations of hexachlorobenzene (HCB), p,p'-dichlorodiphenyl-dichloroethylene (DDE), p,p'-

dichlorodiphenyldichloroethane (DDD), β-HCH, cis-chlordane, trans-nonachlor, cis-nonachlor and heptachlor epoxide. Also no significant location differences were found for adult male liver concentrations of HCB, DDT, photomirex, oxychlordane, cis-chlordane and trans-nonachlor. The hepatic mean of QCB for adult males from WW was higher than means from the other regional locations, but significant only for the OP (means not determined for the LCR, OC and PS because of > 50% non-detects). DDE was higher from the LCR for adult males than the other locations, though significant only for the PS, SW and OC. Adult male DDD means from the LCR and WB were significantly higher than the OC adult mean. The LCR and WW adult means for cis-nonachlor were higher than the OC mean. Juvenile and adult male hepatic mirex means were higher for the LCR than means from all other locations, though only significant for the OP juvenile and the OC and PS adult means (Tables 9 and 10). Adult male β-HCH was significantly higher for the PS than the means from the OC, LCR and WW. The WB juvenile male oxychlordane mean was higher than the other locations, but significant only when compared to the OP. Mean adult male heptachlor epoxide from the WB was higher than the means from OC and SW. Finally, mean dieldrin from the WB was higher than means from the OP, SW and OC for the adult males and OP, OC and WW for juveniles. Overall age comparisons between juvenile and adult males for geometric mean OCs and metabolites are shown in Table 11. Adult means of HCB, DDE, DDD, mirex, oxychlordane, trans-nonachlor and dieldrin were significantly higher than the means for juveniles.

Individual river otter HCB (fungicide and metallurgical fluxing agent) concentrations were generally below 20 μg kg^{-1}, with a few exceptions. Three adult otters (RAG-026, 028, 030) trapped between river miles 111-116 on the Willamette River (near a metallurgical production plant at Millersburg , OR) showed elevated liver HCB concentrations from 22.6 to 572 μg kg^{-1}. Two yearling otters (RAG99-02, RAG99-06) collected on the LCR (river mile 118) at the Chinook Landing boat launch had HCB liver concentrations of 101 and 36.1

μg kg^{-1}, respectively. Three otters (juvenile RAG98-01, yearling RAG99-03, yearling RAG99-04) collected from Portland Harbor (Fremont Bridge) on the Willamette River, OR had liver HCB concentrations of 34.1, 50.0 and 40.0 μg kg^{-1}, respectively. An adult otter (RAG-063) collected south of Hillsboro, OR from a residential pond had 27.6 μg kg^{-1} HCB in its liver. An adult otter (RAG-237) collected near Silverdale, WA off of Dyes Inlet had a liver concentration of 27.0 μg kg^{-1} HCB. A juvenile otter (RAG-159) collected near Centralia, WA had 74.8 μg kg^{-1} HCB in its liver. Finally, juvenile otter (RAG-055) collected off of the Trask River, 3 miles southeast of Tillamook, OR had 32.6 μg kg^{-1} HCB in its liver.

Of the DDTs, only a few otter liver residues were over 1 μg g^{-1}. Otters trapped near the Chinook Landing boat launch (juvenile OT#36, adult OT#37, adult OT#38, yearlings RAG99-02 and RAG99-06) had DDE liver concentrations of ranging from 936 to 1821 μg kg^{-1}. Two otters (juvenile RAG-036, adult RAG-037) collected from the Columbia River near Wenatchee, WA had DDE liver concentrations of 1505 and1360 μg kg^{-1}, respectively. An adult otter (RAG-043) trapped one mile east of Gaston, OR had a DDE liver concentration of 1588 μg kg^{-1}. A surprising liver concentration of 1443 μg kg^{-1} DDD was found for the adult otter (RAG-063) collected south of Hillsboro, OR. The Hillsboro otter had no detectable DDT and only 253 μg kg^{-1} DDE.

PCBs

Mean PCBs for regional locations for are shown in Tables 12 and 13. No statistical comparisons could be made between locations for juvenile male otter liver concentrations of PCBs 31/28, 42, 44, 64, 70, 74, 97, 174 and 185 as these congeners had >50% non-detections (Table 12). The same congeners also could not be compared for the adult males, with the addition of PCB congener 158 which also had >50% non-detections (Table 13). Overall, juvenile males from LCR had the highest hepatic mean ΣPCB concentration, followed by WB, PS, WW, OC, SW and OP, though significant only when compared with SW and OP. Individual juvenile male PCB congener concentrations followed the same

Table 12. Regional location comparisons using geometric mean polychlorinated biphenyl (PCB) residue concentrations (µg kg^{-1}, ww) in livers of juvenile river otter males collected in Oregon and Washington, 1994-99. LCR = Lower Columbia River, OC = Oregon Coast, OP = Olympic Peninsula, PS = Puget Sound, SW = Southwestern Washington, WB = Willamette River Basin, and WW = Western Washington.

Location	**LCR**	**OC**	**OP**	**PS**	**SW**	**WB**	**WW**
n	6	7	7	12	14	8	6
Congener							
ΣPCB	**241 A**	**55.5 ABC**	**32.1 C**	**136 ABC**	**49.1 BC**	**217 AB**	**102 ABC**
PCB 31/28	0.11	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
PCB 42	6 nd	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
PCB 44	0.94	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
PCB 49	0.27 A	7 nd	6 nd	0.11 A	11 nd	6 nd	4 nd
PCB 52	1.82 A	5 nd	0.12 A	0.73 A	0.45 A	0.11 A	0.38 A
PCB 60	0.48 A	0.03 A	0.06 A	0.18 A	0.11 A	0.05 A	0.09 A
PCB 64	0.03	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
PCB 66/95	0.49 A	6 nd	6 nd	0.08 A	10 nd	0.08 A	0.11 A
PCB 70	0.09	8 nd	7 nd	11 nd	13 nd	8 nd	5 nd
PCB 74	0.71	8 nd	7 nd	12 nd	14 nd	8 nd	5 nd
PCB 87	**1.61 A**	**0.02 B**	**0.05 B**	**0.17 AB**	**0.07 AB**	6 nd	**0.12 AB**
PCB 97	4 nd	8 nd	7 nd	12 nd	13 nd	8 nd	6 nd
PCB 99	**14.5 A**	**1.72 BC**	**0.82 C**	**7.35 AB**	**2.53 ABC**	**9.82 AB**	**4.13 ABC**
PCB101	**3.10 A**	**0.10 B**	**0.16 AB**	**1.58 AB**	**0.92 AB**	**0.98 AB**	**0.71 AB**
PCB 105	1.97 A	0.14 A	0.08 A	0.88 A	0.23 A	0.86 A	0.92 A
PCB 110	1.16 A	5 nd	5 nd	0.57 A	0.10 A	0.13 A	0.27 A
PCB 118	**2.94 A**	**0.10 B**	**0.08 B**	**1.25 AB**	**0.52 AB**	**1.41 AB**	**0.58 AB**
PCB 129	1.28 A	0.12 A	4 ND	0.53 A	0.09 A	0.22 A	0.50 A
PCB 138	**59.3 A**	**12.4 B**	**8.77 B**	**39.1 AB**	**13.4 AB**	**57.0 A**	**23.9 AB**
PCB 141	0.25 A	7 nd	7 nd	7 nd	12 nd	6 nd	0.04 A
PCB 146	**4.38 A**	**0.88 BC**	**0.67 C**	**2.90 ABC**	**1.05 ABC**	**3.80 AB**	**1.75 ABC**

Table 12. Continued.

Location	LCR	OC	OP	PS	SW	WB	WW
n	6	7	7	12	14	8	6
Congener							
PCB 149	1.15 A	6 nd	6 ND	0.28 A	0.06 A	0.07 A	0.24 A
PCB 151	0.43 A	8 nd	7 nd	0.08 A	11 nd	5 nd	0.04 A
PCB 153	**39.8 AB**	**9.05 BC**	**7.79 C**	**27.8 ABC**	**10.7 ABC**	**42.0 A**	**18.9 ABC**
PCB 158	1.19 A	7 nd	6 nd	0.09 A	13 nd	0.10 A	0.05 A
PCB 170/190	**19.3 A**	**7.15 AB**	**2.48 B**	**9.51 AB**	**3.43 B**	**18.6 A**	**7.55 AB**
PCB 171	**2.12 A**	**0.28 AB**	**0.06 B**	**0.69 AB**	**0.18 AB**	**1.68 A**	**0.73 AB**
PCB 172	0.76 A	0.07 A	4 nd	0.26 A	0.06 A	0.42 A	0.30 A
PCB 174	5 nd	8 nd	7 nd	12 nd	14 nd	7 nd	5 nd
PCB 180	**30.6 A**	**7.74 AB**	**3.45 B**	**12.4 AB**	**4.62 B**	**28.0 A**	**11.4 AB**
PCB 182/187	**14.6 A**	**2.65 ABC**	**1.98 C**	**8.54 ABC**	**2.49 BC**	**12.1 AB**	**7.31 ABC**
PCB 183	**5.04 A**	**0.95 ABC**	**0.65 C**	**2.70 ABC**	**0.92 BC**	**4.18 AB**	**1.95 ABC**
PCB 185	6 nd	8 nd	7 nd	12 nd	14 nd	8 nd	6 nd
PCB 194	**4.28 A**	**1.45 AB**	**0.09 B**	**1.46 AB**	**0.23 AB**	**4.19 A**	**1.89 A**
PCB 195	**2.17 A**	**0.59 AB**	**0.05 B**	**0.56 AB**	**0.10 B**	**1.04 AB**	**0.74 AB**
PCB 200	**1.68 A**	**0.03 B**	**0.04 B**	**0.49 AB**	**0.09 AB**	**1.01 A**	**0.51 AB**
PCB 201	**11.5 A**	**2.54 ABC**	**0.90 C**	**6.14 AB**	**1.56 BC**	**9.36 A**	**5.76 AB**
PCB 203	**4.13 A**	**0.76 AB**	**0.39 B**	**1.91 AB**	**0.45 B**	**3.45 A**	**1.80 AB**
PCB 206	**3.17 A**	**0.56 AB**	**0.11 B**	**1.27 AB**	**0.34 AB**	**2.74 A**	**1.84 A**

One-way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows of the categories sharing the same letters are not significantly different. # nd = number of samples with non detections.

Table 13. Regional location comparisons using geometric mean polychlorinated biphenyl (PCB) residue concentrations (µg kg^{-1}, ww) in livers of adult male river otters collected in Oregon and Washington, 1994-99. LCR = Lower Columbia River, OC = Oregon Coast, OP = Olympic Peninsula, PS = Puget Sound, SW = Southwestern Washington, WB = Willamette River Basin, and WW = Western Washington.

Location	**LCR**	**OC**	**OP**	**PS**	**SW**	**WB**	**WW**
n	9	17	18	12	16	17	5
Congener							
∑PCB	**564 A**	**96.6 B**	**90.4 B**	**585 A**	**78.9 B**	**219 AB**	**322 A**
PCB 31/28	0.12	17 nd	18 nd	12 nd	16 nd	16 nd	5 nd
PCB 42	9 nd	17 nd	18 nd	12 nd	16 nd	17 nd	5 nd
PCB 44	0.78	15 nd	18 nd	12 nd	14 nd	14 nd	5 nd
PCB 49	**0.63 A**	**16 nd**	**0.06 B**	**8 nd**	**11 nd**	**12 nd**	**0.08 AB**
PCB 52	**3.12 A**	**0.11 C**	**0.52 ABC**	**1.22 AB**	**0.39 ABC**	**0.20 BC**	**1.11 ABC**
PCB 60	**1.31 A**	**0.09 B**	**0.45 AB**	**0.27 AB**	**0.30 AB**	**11 nd**	**0.64 AB**
PCB 64	5 nd	17 nd	18 nd	12 nd	16 nd	16 nd	5 nd
PCB 66/95	**1.42 A**	**0.03 B**	**0.11 AB**	**0.19 AB**	**0.03 B**	**0.22 AB**	**0.29 AB**
PCB 70	0.22	16 nd	16 nd	12 nd	15 nd	16 nd	5 nd
PCB 74	0.13	16 nd	17 nd	11 nd	16 nd	16 nd	5 nd
PCB 87	**2.58 A**	**10 nd**	**0.19 B**	**0.35 AB**	**0.05 B**	**13 nd**	**0.33 AB**
PCB 97	7 nd	17 nd	17 nd	12 nd	16 nd	16 nd	5 nd
PCB 99	**29.3 A**	**5.16 B**	**5.11 B**	**25.6 A**	**3.41 B**	**9.33 AB**	**14.9 AB**
PCB 101	**4.89 A**	**0.35 D**	**0.76 BCD**	**2.80 AB**	**0.46 CD**	**1.32 ABCD**	**2.14 ABC**
PCB 105	**3.04 A**	**0.08 B**	**0.26 AB**	**0.43 AB**	**0.19 AB**	**9 nd**	**0.57 AB**
PCB 110	**1.67 A**	**0.05 B**	**0.18 AB**	**0.45 AB**	**0.08 B**	**9 nd**	**0.38 AB**
PCB 118	**3.41 A**	**0.27 B**	**0.66 AB**	**1.29 AB**	**0.41 B**	**0.55 AB**	**1.37 AB**
PCB 129	**2.33 A**	**0.18 BC**	**0.19 BC**	**2.73 A**	**0.21 BC**	**0.07 C**	**1.07 AB**
PCB 138	**155 A**	**23.9 B**	**26.2 B**	**145 A**	**20.9 B**	**60.2 AB**	**85.6 A**
PCB 141	0.58 A	16 nd	13 nd	7 nd	13 nd	14 nd	0.08 A
PCB 146	**8.66 A**	**1.17 D**	**1.36 CD**	**7.93 AB**	**0.92 D**	**3.00 BCD**	**5.10 ABC**

Table 13. Continued.

Location	LCR	OC	OP	PS	SW	WB	WW
n	9	17	18	12	16	17	5
Congener							
PCB 149	**1.86 A**	**0.05 C**	**0.30 ABC**	**0.26 ABC**	**0.06 BC**	**0.10 BC**	**0.84 AB**
PCB 151	0.48 A	13 nd	0.04 A	0.08 A	13 nd	14 nd	0.08 A
PCB 153	**98.4 A**	**19.3 C**	**22.6 BC**	**126 A**	**18.6 C**	**43.4 ABC**	**69.6 AB**
PCB 158	2.56	16 nd	17 nd	7 nd	16 nd	12 nd	3 nd
PCB 170/190	**57.1 AB**	**9.50 CDE**	**6.47 E**	**62.9 A**	**6.77 DE**	**20.2 BCD**	**26.5 ABC**
PCB 171	**2.75 A**	**0.35 B**	**0.35 B**	**3.58 A**	**0.31 B**	**1.32 AB**	**2.01 A**
PCB 172	**1.29 A**	**0.16 BC**	**0.13 C**	**1.19 A**	**0.13 C**	**0.18 BC**	**0.71 AB**
PCB 174	5 nd	17 nd	16 nd	10 nd	16 nd	16 nd	5 nd
PCB 180	**85.0 A**	**1.37 B**	**9.89 B**	**71.6 A**	**10.3 B**	**31.1 AB**	**47.1 A**
PCB 182/187	**27.6 A**	**3.67 B**	**3.36 B**	**21.3 A**	**3.00 B**	**9.09 AB**	**16.2 A**
PCB 183	**12.4 A**	**1.51 B**	**1.56 B**	**11.8 A**	**1.46 B**	**4.13 AB**	**6.86 A**
PCB 185	9 nd	16 nd	18 nd	12 nd	16 nd	17 nd	5 nd
PCB 194	**10.6 A**	**2.11 BCD**	**1.27 D**	**13.3 A**	**1.44 DC**	**4.16 ABC**	**6.08 AB**
PCB 195	**4.98 A**	**0.42 BC**	**0.30 C**	**4.69 A**	**0.34 BC**	**1.57 AB**	**2.04 A**
PCB 200	**3.08 AB**	**0.48 DC**	**0.32 D**	**3.28 A**	**0.31 D**	**0.93 BCD**	**1.52 ABC**
PCB 201	**21.90 A**	**2.60 DC**	**1.74 D**	**25.13 A**	**1.82 D**	**5.33 BC**	**9.88 AB**
PCB 203	**9.51 A**	**1.02 B**	**0.84 B**	**8.57 A**	**0.87 B**	**3.69 A**	**5.09 A**
PCB 206	**6.72 AB**	**1.42 CD**	**0.87 D**	**12.9 A**	**1.03 D**	**3.10 BC**	**4.08 B**

One-way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows of the categories sharing the same letters are not significantly different. # nd number of samples with non detections.

Table 14. Age comparisons using geometric mean PCB concentrations (μg kg^{-1}, ww) in livers of juvenile and adult river otter males collected in Oregon and Washington, 1994-99.

	Juvenile (n = 60)			Adult (n = 94)		
Congener	Mean	Range	sd	Mean	Range	sd
ΣPCBs	**89.3 B**	10.2 - 1857	0.49	**170 A**	16.1 - 10087	0.49
PCB 31/28		56nd - 0.87			86nd - 1.24	
PCB 42		60nd			94nd	
PCB 44		54nd -1.21			78nd - 8.74	
PCB 49		39nd - 2.17			58nd - 9.15	
PCB 52	0.27 A	13nd - 13.5	1.02	0.44 A	13nd - 29.9	0.89
PCB 60	**0.10 B**	21nd - 2.98	0.99	**0.21 A**	21nd - 9.11	0.93
PCB 64		56nd - 0.09			89nd - 0.54	
PCB 66/95		33nd - 3.85		0.94	46nd - 15.5	0.99
PCB 70		53nd - 0.60			81nd - 2.26	
PCB 74		53nd - 0.98			84nd - 4.16	
PCB 87	0.08 A	28nd - 5.09	1.07	0.10 A	36nd - 10.1	1.10
PCB 97		57nd - 0.44			90nd - 0.48	
PCB 99	**3.93 B**	1nd - 60.7	0.64	**8.19 A**	1nd - 375	0.59
PCB 101	0.71 A	8nd - 10.7	0.91	1.00 A	2nd - 33.0	0.66
PCB 105	0.43 A	13nd - 10.8	1.07	0.21 A	29nd - 28.7	1.17
PCB 110	0.14 A	21nd - 3.07	1.06	0.15 A	30nd - 10.3	1.07
PCB 118	0.57 A	9nd - 5.50	0.93	0.66 A	7nd - 22.4	0.73
PCB 129	0.20 A	15nd - 13.5	1.01	0.32 A	10nd - 35.4	0.82
PCB 138	**23.3 B**	2.30 - 458	0.50	**45.3 A**	3.42 - 2229	0.49
PCB 141		42nd - 1.52			66nd - 5.88	
PCB 146	1.73 A	0.24 - 42.7	0.50	2.18 A	1nd - 109	0.57
PCB 149	0.09 A	24nd - 4.87	1.10	0.17 A	26nd - 15.0	1.02
PCB 151		40nd - 2.67			54nd - 13.5	
PCB 153	**17.7 B**	1.92 - 284	0.48	**36.4 A**	2.86 - 2782	0.49
PCB 158		38nd - 10.4			71nd - 9.19	
PCB 170/190	**7.11 B**	0.60 - 192	0.51	**15.2 A**	1.82 - 939	0.53
PCB 171	**0.43 B**	7nd - 22.7	0.89	**0.78 A**	2nd - 93.6	0.68
PCB 172	**0.14 B**	14nd - 9.85	0.89	**0.26 A**	5nd - 20.6	0.66
PCB 174		57nd - 0.58			86nd - 0.99	
PCB 180	**9.72 B**	0.56 - 259	0.53	**22.4 A**	2.76 - 1829	0.52
PCB 182/187	5.13 A	0.55 - 157	0.56	6.64 A	0.95 - 331	0.53
PCB 183	**1.72 B**	0.22 - 48.9	0.53	**3.13 A**	0.36 - 227	0.56
PCB 185		60 nd			93nd - 0.19	
PCB 194	**0.89 B**	8nd - 36.4	0.98	**3.17 A**	0.40 - 428	0.54
PCB 195	**0.36 B**	10nd - 23.0	0.97	**0.91 A**	3nd - 82.3	0.70

Table 14. Continued.

	Juvenile (n = 60)			Adult (n = 94)		
Congener	Mean	Range	sd	Mean	Range	sd
PCB 200	**0.23 B**	15nd - 6.42	0.98	**0.76 A**	1nd - 28.3	0.57
PCB 201	3.60 A	0.30 - 123	0.55	4.54 A	0.65 - 242	0.56
PCB 203	**1.18 B**	1nd - 41.6	0.62	**2.13 A**	0.20 - 164	0.60
PCB 206	**0.81 B**	5nd - 14.6	0.80	**2.29 A**	0.29 - 309	0.53

One-way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows sharing the same letters are not significantly different. # nd = number of samples with non-detections. sd = standard deviation. Means and sd not calculated if non-detections > 50% of n.

general pattern as the ΣPCBs (Table 12). Adult males, however, were different in that the ΣPCB concentrations for the PS, LCR and WW were significantly higher than OC, OP and SW.

Overall age comparisons between juvenile and adult males for geometric mean PCBs are shown in Table 14. Mean hepatic PCBs were always higher for adult males than juveniles, except for PCB 105. Significant differences were with the ΣPCBs and individual congeners 60, 99, 138, 153, 170/190, 171, 172, 180, 183, 194, 195, 200, 203 and 206. PCB means for both were low overall, though extreme individual concentrations for the ΣPCBs and congeners PCB 138, 153 and 180 did exceed 1 μg g^{-1}. Six otters (yearling OT#35, juvenile OT#36, adult OT#37, adult OT#38, yearling RAG99-02, yearling RAG99-06) collected from the lower Columbia River between river mile 117-120 near Troutdale, OR had hepatic ΣPCB concentrations ranging from 926 to 2414 μg kg^{-1}. One juvenile otter (RAG98-01) from the Portland Harbor at Fremont Bridge and three yearling otters (RAG-025, RAG99-03, RAG99-04) had liver ΣPCB concentrations ranging from 918 to 3573 μg kg^{-1}. An adult otter (OT#34) trapped from Scappoose Bay near the mouth of Multnomah channel (LCR river mile 88) had a hepatic ΣPCB concentration of 1055 μg kg^{-1}. An adult otter (RAG-037) trapped on the Columbia River near Wenatchee, WA had a ΣPCB concentration of 1070 μg kg^{-1}. Two adult otters (RAG-080, RAG-081) trapped from Bear Creek (a tributary of the Sammamish River), just north of Woodinville, WA had ΣPCB

concentrations of 1620 and 1183 μg kg^{-1}, respectively. Finally, three otters from Dyes Inlet northwest of Bremerton, WA (yearling RAG-203, adult RAG-193, adult RAG-237) in the Puget Sound had ΣPCB concentrations of 958, 3794 and 10,087 μg kg^{-1}, respectively, with the highest ΣPCB concentrations found in this study.

Profile patterns of PCBs for the 7 regional locations are shown in Figures 28 and 29. Juvenile male otter congener profiles were similar for all locations, with PCB-38 having the greatest percentage contribution, followed by PCBs 153 and 180. Congener profiles for the adults were similar to the juvenile profiles except for the PS which had PCB-153 as the congener with the greatest percentage contribution rather than PCB-138.

Non-ortho Substituted PCBs, Dioxins and Furans

Geometric mean juvenile and adult liver concentrations of non-ortho substituted PCBs, dioxins and furans were quite low for all locations (Tables 15 and 16). No statistical comparisons could be made between locations for mean juvenile male otter liver concentrations of TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (12378-PCDD), total PCDD, 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (123478-H6CDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 1,2,3,7,8-pentachlorodibenzofuran (12378-PCDF), 1,2,3,6,7,8-hexachlorodibenzofuran (123678-H6CDF), 123789-H6CDF and 1,2,3,4,7,8,9-heptachlorodibenzofuran (1234789-H7CDF) as these congeners had >50% non-detections for each location. The same was true for comparisons between locations for adult males including 123789-H6CDD, but TCDF comparisons could be made between two locations (LCR and WW). No significant differences in mean liver concentrations were found between locations for juvenile and adult male non-ortho substituted PCBs 77, 126 and 169, except for PCB-126 where adults from the LCR were higher than from WB and OC (Tables 15 and 16). No significant differences were found between juvenile and adult means for the non-ortho substituted PCBs (Table 17).

No significant differences in juvenile geometric liver means were found

between regional locations for total TCDD, 123789-H6CDD, octachlorodibenzo-*p*-dioxin (OCDD), total TCDF, 12478-PCDF, total PCDF, 123478-H6CDF, 234678-H6CDF, total H6CDF, 1234678-H7CDF, total H7CDF and octachlorodibenzofuran (OCDF) (Table 15). No significant adult mean differences were found between locations for total TCDD, 123678-H6CDD, TCDF, total TCDF, 12478-PCDF, total PCDF, 123478-H6CDF and 234678-H6CDF (Table 16). However, 123678-H6CDD and total H6CDD in juveniles from the LCR were higher than at other locations, but significant only with OP. Mean juvenile 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (1234678-H7CDD) was significantly higher at locations LCR, OC, WB and PS compared to OP, with total H7CDD at LCR higher compared to SW and OP. Mean adult male total H6CDD at LCR and PS were significantly higher than at SW and OP. Adult male 1234678-H7CDD and total H7CDD liver means were significantly higher for the LCR, PS and WB locations when compared to SW and OP; mean PS OCDD was higher than the other locations, but significant only when compared with SW and OP. Adult male mean total H6CDF for the LCR was higher than all other locations, but significant only when compared with OP. Adult mean 1234678-H7CDF was significantly higher for LCR and PS compared to WW and SW; mean total H7CDF higher at PS than OC and SW; and OCDF higher at PS and LCR compared to SW.

Overall, no significant differences were found between juvenile and adult geometric mean hepatic concentrations of non-ortho substituted PCB, dioxin and furan concentrations (Table 17). Juvenile male hepatic means for dioxins were always higher than the adults, but this was reversed for the furans. Again, concentration means of non-ortho substituted PCB, dioxins and furans were quite low for each age group. Often the LCR had the highest concentrations, followed by PS, although the differences were not always significant.

Non-ortho substituted PCB congeners of individual otters were generally quite low with only a few otters having hepatic concentrations above 100 ng kg^{-1}.

Table 15. Regional location comparisons using geometric mean non-ortho substituted PCB, PCDD, PCDF residue concentrations (ng kg^{-1}, ww) and TEQs (ng kg^{-1}, ww) in livers of juvenile river otter males collected in Oregon and Washington, 1994-99. LCR = Lower Columbia River, OC = Oregon Coast, OP = Olympic Peninsula, PS = Puget Sound, SW = Southwestern Washington, WB = Willamette River Basin, and WW = Western Washington.

Location	**LCR**	**OC**	**OP**	**PS**	**SW**	**WB**	**WW**
n	5	7	7	12	14	8	6
Congener							
PCB 77	2.39 A	4.27 A	4.00 A	2.15 A	3.98 A	1.75 A	3.62 A
PCB 81	0.15A	6 nd	7 nd	9 nd	11 nd	7 nd	0.07A
PCB 126	27.6 A	6.93 A	6.74 A	21.8 A	3.16 A	9.99 A	16.5 A
PCB 169	9.10 A	3.48 A	2.14 A	4.91 A	3.99 A	3.40 A	5.07 A
2378-TCDD	1.03	6 nd	7 nd	9 nd	13 nd	5 nd	6 nd
Total TCDD	1.03 A	0.12 A	5 nd	0.12 A	13 nd	0.06 A	5 nd
12378-PCDD	0.16	7 nd	7 nd	10 nd	14 nd	7 nd	6 nd
Total PCDD	0.16	7 nd	7 nd	9 nd	14 nd	7 nd	6 nd
123478-H6CDD	4 nd	7 nd	6 nd	11 nd	13 nd	8 nd	6 nd
123678-H6CDD	**14.2 A**	**5.62 AB**	**0.10 B**	**1.90 AB**	**0.24 AB**	**1.41 AB**	**4.86 AB**
123789-H6CDD	0.41 A	6 nd	7 nd	0.06 A	12 nd	6 nd	4 nd
TotalH6CDD	**16.5 A**	**5.90 AB**	**0.12 B**	**1.37 AB**	**1.20AB**	**3.94 AB**	**5.13 AB**
1234678-H7CDD	**69.1 A**	**29.7 A**	**1.14 B**	**20.2 A**	**4.97 AB**	**26.7 A**	**12.7 AB**
Total H7CDD	**114 A**	**31.2 AB**	**1.14 C**	**21.3 AB**	**5.67 BC**	**32.6 AB**	**13.6 ABC**
OCDD	139 A	89.5 A	13.7 A	70.0 A	13.9 A	56.0 A	20.0 A
2378-TCDF	0.08	6 nd	6 nd	9 nd	10 nd	7 nd	5 nd
Total TCDF	0.16 A	6 nd	4 nd	0.19 A	0.18 A	7 nd	4 nd
12378-PCDF	3 nd	7 nd	7 nd	12 nd	14 nd	8 nd	5 nd
12478-PCDF	4.18 A	5 nd	7 nd	7 nd	12 nd	6 nd	0.30 A
Total PCDF	6.50 A	0.34 A	6 nd	0.61 A	10 nd	5 nd	0.36 A
123478-H6CDF	6.55 A	0.22 A	6 nd	0.16 A	0.41 A	1.52 A	0.92 A
234678-H6CDF	1.95 A	0.17 A	6 nd	0.10 A	0.13 A	0.19 A	0.07 A

Table 15. Continued.

Location	LCR	OC	OP	PS	SW	WB	WW
n	5	7	7	12	14	8	6
Congener							
123678-H6CDF	1.51	7 nd	7 nd	10 nd	13 nd	8 nd	6 nd
123789-H6CDF	4 nd	7 nd	7 nd	11 nd	14 nd	8 nd	6 nd
Total H6CDF	10.8 A	1.58 A	5 nd	0.78 A	0.64 A	5.05 A	3.74 A
1234678-H7CDF	15.0 A	0.95 A	0.15 A	1.08 A	0.17 A	1.43 A	0.39 A
1234789-H7CDF	5 nd	6 nd	7 nd	10 nd	13 nd	7 nd	5 nd
Total H7CDF	17.5 A	2.64 A	0.16 A	2.94 A	0.24 A	1.51 A	0.90 A
OCDF	3.85 A	0.89 A	7 nd	0.18 A	8 nd	0.65 A	0.08 A
PCB TEQs	**3.32 A**	**0.91 AB**	**0.77 C**	**2.57 ABC**	**0.80 BC**	**1.41 AB**	**1.92 ABC**
Dioxin TEQs	**4.74 A**	**0.91 AB**	**0.06 B**	**0.87 AB**	**0.25 B**	**0.89 AB**	**0.64 AB**
Furan TEQs	**3.45 A**	**0.18 AB**	**0.01 B**	**0.43 AB**	**0.14 AB**	**0.26 AB**	**1.07 A**
Total TEQs	**12.5 A**	**2.63 AB**	**1.04 B**	**4.96 AB**	**2.05 AB**	**3.47 AB**	**4.06 AB**

One-way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows of the categories sharing the same letters are not significantly different. # nd = number of samples with non detections. PCB = polychlorinated biphenyl, TCDD = tetrachlorodibebzo-p-dioxin, PCDD = pentachlorodibenzo-p-dioxin, H6CDD = hexachlorodibenzo-p-dioxin, H7CDD = heptachlorodibenzo-p-dioxin, OCDD = octachlordibenzo-p-dioxin, TCDF = tetrachlorodibenzofuran, PCDF = pentachlorodibenzofuran, H6CDF = hexachlorodibenzofuran, H7CDF = heptachlorodibenzofuran, OCDF = octachlordibenzofuran. TEQ = toxic equivalents.

Table 16. Regional location comparisons using geometric mean non-ortho substituted PCB, PCDD, PCDF residue concentrations (ng kg^{-1}, ww) and TEQs (ng kg^{-1}, ww) in livers of adult male river otters collected in Oregon and Washington, 1994-99. LCR = Lower Columbia River, OC = Oregon Coast, OP = Olympic Peninsula, PS = Puget Sound, SW = Southwestern Washington, WB = Willamette River Basin, and WW = Western Washington.

Location	**LCR**	**OC**	**OP**	**PS**	**SW**	**WB**	**WW**
n	9	17	18	12	16	17	5
Congener							
PCB 77	3.84 A	0.64 A	1.61 A	2.39 A	4.51	2.64 A	0.84 A
PCB 81	0.22	12 nd	14 nd	9 nd	16 nd	13 nd	3 nd
PCB 126	**50.9 A**	**1.60 C**	**6.54 ABC**	**31.6 AB**	**5.31 ABC**	**3.33 BC**	**15.8 ABC**
PCB 169	18.4 A	4.99 A	4.60 A	13.7 A	3.47 A	5.64 A	7.23 A
2378-TCDD	0.83	10 nd	16 nd	8 nd	12 nd	11 nd	3 nd
Total TCDD	0.90 A	0.21 A	11 nd	0.64 A	0.29 A	0.31 A	0.49 A
12378-PCDD	0.23	10 nd	17 nd	12 nd	16 nd	15 nd	4 nd
Total PCDD	0.23	9 nd	17 nd	12 nd	16 nd	13 nd	4 nd
123478-H6CDD	7 nd	15 nd	16 nd	12 nd	15 nd	16 nd	5 nd
123678-H6CDD	11.0 A	0.49 A	11 nd	9.21 A	10 nd	1.00 A	0.31 A
123789-H6CDD	0.43	9 nd	18 nd	7 nd	14 nd	11 nd	3 nd
Total H6CDD	**12.9 A**	**1.05 AB**	**0.19 B**	**11.1 A**	**0.28 B**	**4.41 AB**	**1.42 AB**
1234678-H7CDD	**43.8 A**	**9.96 AB**	**0.23 C**	**37.4 A**	**1.23 BC**	**28.9 A**	**10.4 AB**
TotalH7CDD	**59.8 A**	**10.0 AB**	**0.67 C**	**41.4 A**	**2.16 BC**	**30.4 A**	**11.1 AB**
OCDD	**112 AB**	**13.5 ABC**	**3.38 C**	**138 A**	**13.3 BC**	**54.7 AB**	**20.8 ABC**
2378-TCDF	0.10 A	13 nd	12 nd	12 nd	15 nd	15 nd	0.10 A
Total TCDF	1.35 A	11 nd	0.23 A	10 nd	0.09 A	12 nd	0.42 A
12378-PCDF	5 nd	17 nd	18 nd	12 nd	16 nd	17 nd	5 nd
12478-PCDF	4.19 A	0.16 A	10 nd	0.25 A	0.17 A	13 nd	0.19 A
Total PCDF	6.34 A	0.27 A	10 nd	1.89 A	0.28 A	10 nd	0.95 A
123478-H6CDF	7.10 A	0.74 A	9 nd	0.76 A	0.39 A	1.99 A	0.82 A
234678-H6CDF	0.83 A	0.07 A	16 nd	0.19 A	10 nd	0.36 A	3 nd

Table 16. Continued.

Location	LCR	OC	OP	PS	SW	WB	WW
n	9	17	18	12	16	17	5
Congener							
123678-H6CDF	1.32	9 nd	18 nd	11 nd	14 nd	15 nd	4 nd
123789-H6CDF	8 nd	17 nd	18 nd	12 nd	16 nd	17 nd	4 nd
Total H6CDF	**11.6 A**	**1.28 AB**	**0.18 B**	**2.30 AB**	**0.80 AB**	**2.30 AB**	**1.31 AB**
1234678-H7CDF	**13.3 A**	**0.31 AB**	**13 nd**	**13.3 A**	**0.16 B**	**3.05 AB**	**0.27 B**
1234789-H7CDF	9 nd	17 nd	18 nd	9 nd	14 nd	12 nd	0.10
Total H7CDF	**14.0 AB**	**0.31 BC**	**12 nd**	**15.5 A**	**0.16 C**	**3.10 ABC**	**2.33 ABC**
OCDF	**1.63 A**	**0.11 AB**	**14 nd**	**1.99 A**	**0.03 B**	**0.13 AB**	**0.16 AB**
PCB TEQs	**6.08 A**	**0.58 C**	**0.88 BC**	**3.84 A**	**0.90 BC**	**0.74 BC**	**2.02 AB**
Dioxin TEQs	**3.15 A**	**0.51 AB**	**0.05 B**	**1.86 A**	**0.09 B**	**1.37 A**	**1.24 A**
Furan TEQs	**3.52 A**	**0.33 AB**	**0.12 B**	**1.72 AB**	**0.26 AB**	**0.37 AB**	**1.19 AB**
Total TEQs	**13.3 A**	**1.93 DC**	**1.78 D**	**8.49 AB**	**2.07 DC**	**3.46 BCD**	**5.31 ABC**

One-way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows of the categories sharing the same letters are not significantly different. # nd = number of samples with non detections. PCB = polychlorinated biphenyl, TCDD = tetrachlorodibebzo-p-dioxin, PCDD = pentachlorodibenzo-p-dioxin, H6CDD = hexachlorodibenzo-p-dioxin, H7CDD = heptachlorodibenzo-p-dioxin, OCDD = octachlordibenzo-p-dioxin, TCDF = tetrachlorodibenzofuran, PCDF = pentachlorodibenzofuran, H6CDF = hexachlorodibenzofuran, H7CDF = heptachlorodibenzofuran, OCDF = octachlordibenzofuran. TEQs = toxic equivalents.

Table 17. Age comparisons using geometric mean co-planar PCB, PCDD and PCDF concentrations (ng kg^{-1}, ww) and TEQs (ng kg^{-1}, ww) in livers of juvenile and adult river otter males collected in Oregon and Washington, 1994-99.

	Juvenile (n = 59)			Adult (n = 94)		
Congener	**Mean**	**Range**	**sd**	**Mean**	**Range**	**sd**
PCB 77	3.01 A	1nd - 36.7	0.62	1.96 A	13nd - 108	0.92
PCB81		44nd - 10.7			62nd - 27.2	
PCB126	9.33 A	2nd - 81.3	0.70	6.75 A	6nd - 380	0.93
PCB169	4.09 A	1nd - 46.4	0.52	6.20 A	2nd - 169	0.63
2378-TCDD		45nd - 506			61nd - 3.22	
Total TCDD		35nd - 506		0.23	27nd - 43.2	1.13
12378-PCDD		53nd - 6.60			76nd - 6.37	
Total PCDD		52nd - 6.88			73nd - 6.37	
123478-H6CDD		56nd - 31.5			86nd - 17.4	
123678-H6CDD	1.17 A	12nd - 154	1.28	0.46 A	28nd - 83.1	1.49
123789-H6CDD		42nd - 8.60			65nd - 5.02	
Total H6CDD	1.93 A	9nd - 208	1.19	1.37 A	24nd - 96.1	1.29
1234678-H7CDD	11.9 A	2nd - 344	0.85	5.63 A	10nd - 326	1.24
Total H7CDD	13.4 A	0.01 - 1757	0.89	8.04 A	6nd - 426	1.08
OCDD	36.6 A	0.01 - 1521	0.81	22.4 A	2nd - 1403	0.94
2378-TCDF		45nd - 17.4			72nd - 23.1	
Total TCDF		32nd - 23.3			52nd - 32.8	
12378-PCDF		56nd - 6.23			90nd - 3.56	
12478-PCDF		39nd - 18.4		0.14	42nd - 22.6	1.33
Total PCDF	0.15 A	28nd - 19.8	1.50	0.31 A	29nd - 25.3	1.31
123478-H6CDF	0.33 A	22nd - 197	1.46	0.58 A	25nd - 66.8	1.37
234678-H6CDF	0.12 A	25nd - 9.10	1.19	0.08 A	47nd - 6.87	1.21
123678-H6CDF		51nd - 5.09			71nd - 11.1	
123789-H6CDF		59nd - 0.19			92nd - 0.25	
Total H6CDF	0.99 A	11nd - 206	1.27	1.20 A	17nd - 80.0	1.24
1234678-H7CDF	0.64 A	21nd - 209	1.54	0.57 A	31nd - 204	1.56
1234789-H7CDF		47nd - 5.78			81nd - 8.49	
Total H7CDF	1.07 A	13nd - 209	1.42	0.75 A	24nd - 204	1.55
OCDF	0.14 A	28nd - 69.0	1.41	0.11 A	41nd - 42.9	1.34
PCB TEQs	1.36 A	0.05 - 9.23	0.41	1.20 A	0.01 - 40.8	0.51
Dioxin TEQs	0.54 A	0.001 - 507	0.89	0.42 A	0.001 - 20.4	1.04
Furan TEQs	0.23 A	0.001 - 31.9	1.21	0.44 A	0.001 - 20.7	1.09
Total TEQs	3.13 A	0.08 - 508	0.59	3.28 A	0.15 - 74.5	0.46

One-way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows sharing the same letters are not significantly different. # nd = number of samples with non-detections. Means and standard deviations (sd) were not calculated if non-detections > 50% of n.

Two otters (adult OT# 34, adult OT# 38) trapped near Chinook Landing boat launch on the LCR had PCB-126 liver concentrations of 147 and 380 ng kg^{-1}, respectively. Otter OT# 38 plus OT# 37 from the same location had PCB-169 liver concentrations of 166 and 125 ng kg^{-1}, respectively. Adult male otter RAG-237 from Silverdale, WA had a PCB-169 liver concentration of 131 ng kg^{-1}.

Individual liver concentrations of dioxins were found in the low ng kg^{-1} range for most river otters collected from Oregon and Washington. Twenty-six of 195 otters (6 juvenile, 3 yearling, 17 adult) had liver TCDD concentrations above 1 ng kg^{-1}, with only two above 5 ng kg^{-1}. Yearling male RAG-025 trapped at Portland harbor near the Fremont Bridge on the Willamette River had a hepatic TCDD concentration of 8.13 ng kg^{-1}, with 75.3, 382 and 995 ng kg^{-1} for total H6CDD, total H7CDD and OCDD, respectively. Juvenile male otter RAG-204 collected at Miller Swamp, 19 km (12 miles) west of Chehalis, WA along the Chehalis River had 506 ng kg^{-1} of TCDD and 1521 ng kg^{-1} OCDD in its liver. Several otter collection sites had high total H7CDD and OCDD concentrations. Only 12 of 191 otters had TCDF concentrations above 1 ng kg^{-1}, with nine above 5 ng kg^{-1} (5.38 - 23.1 ng kg^{-1}).

All but two of the 12 otters having TCDF above 1 ng kg^{-1} were from the less populated areas of the OP and SW. Two otters (yearling OT# 15, adult OT# 34) collected between river mile 86.9 - 88.0 on the LCR near St. Helens, OR had liver concentrations of 983 and 426 ng kg^{-1} total H7CDD and 4518 and 1255 ng kg^{-1} OCDD, respectively. Adult otter OT# 18 collected at river mile 39.1 on the LCR at the lower end of Puget Island had liver concentrations of 410 ng kg^{-1} for total H7CDD and 1183 ng kg^{-1} for OCDD. Yearling otter (RAG-085) collected near Newberg, OR just off of the Willamette River also had elevated total H7CDD and OCDD concentrations at 631 and 1286 ng kg^{-1}, respectively. Four otters (yearling RAG-025, juvenile RAG98-01, yearling RAG99-03, yearling RAG99-04) collected near the Fremont Bridge on the Willamette River at Portland Harbor, OR had total H7CDD concentrations ranging from 324 - 750 ng kg^{-1} and OCDD from 995-3429 ng kg^{-1}. Otter RAG-025 from Portland Harbor

also had a liver concentration of 1213 ng kg^{-1} for 123478-H6CDF. Yearling otters RAG99-03 and OT#15 also had hepatic concentrations of 954 and 707 ng kg^{-1} total H7CDF and 315 and 920 ng kg^{-1} OCDF. Three otters (adult RAG-007, juveniles RAG-009 and RAG-010) collected from the Fiddle Creek inlet of coastal Siltcoos Lake, south of Florence, OR had total H7CDD and OCDD liver concentrations ranging from 138 - 326 and 410 - 1404 ng kg^{-1}, respectively. Total H7CDD and OCDD concentrations found at Siltcoos Lake were surprising as no industry was associated with location. However, a railroad trestle crosses through Siltcoos Lake at several locations, with the trestle wood treated with pentachlorophenol (PCP).

TEQs

The additive TEQs (ng kg^{-1}, ww) for PCBs, PCDDs, PCDFs and total TEQs were evaluated as residue concentrations and results are shown in Tables 15 and 16. Geometric mean TEQ values were low for both otter age groups, with LCR otters having TEQ means above 10 ng kg^{-1} for both juveniles and adults. No pattern of TEQ increase associated with age was evident. In general, mean TEQs for the LCR were always higher than other locations for both age groups. However, juvenile TEQs for LCR were significantly higher than locations OP (all TEQ means) and SW (PCB and dioxin TEQ means). Adult TEQ means for the LCR were only significantly higher than locations OC (PCB and total TEQ means), OP (all TEQ means), SW (PCB, dioxin and total TEQ means) and WB (PCB and total TEQ means).

No significant TEQ differences were found between juveniles and adults (Table 17). Only 2 total TEQ values were over 100 ng kg^{-1} for individual otters. Yearling otter RAG-025 from Portland Harbor had a total TEQ of 180 ng kg^{-1}. The highest total TEQ was 508 ng kg^{-1} for a juvenile male otter (RAG-204) which also had the high TCDD mentioned above.

Butyltins

Butyltin compounds were found in all 40 river otter liver samples analyzed (Table 18). Liver concentrations from locations in Oregon ranged from 8.50 to 102 ng g^{-1} (ww), with samples from Washington ranging from 11.0 to 2,610 ng g^{-1} (ww). Table 19 shows regional geometric mean comparisons between OC, OP, PS, SW and Willamette River (W) for MBT, DBT, TBT and butyltins (BTs). Butyltin residues of the otter collected from LCR at Scappoose Bay, Oregon was combined with otters from the W. The PS region had the highest geometric mean liver concentrations for all butyltins when compared to other regions. DBT and BTs from PS were significantly higher compared to other regions, with MBT from PS significantly higher than OC and OP. No significant differences were found between locations for mean TBT residues, though PS was highest. The greatest concentration of BTs (2,610 ng g^{-1}, ww) was found in a otter collected from Fort Ward, Washington off of the southern end of Bainbridge Island. Otter concentrations of BTs >1000 ng g^{-1} (ww) were found in individual otters from Camano Island, Bremerton and Eglon, all in the PS region. However, butyltins were found in fresh water rivers and streams as well.

Perfluorochemicals

Perfluorochemicals were found in all 20 otter liver samples analyzed (Table 20). PFOS was found in higher concentrations than the other perfluorochemicals, with FOSA ranking second. Residues from otters collected at two coastal rivers in OC and OP were combined for PFOS and FOSA to compare with residue means from PS and W. Greater than 50% of the PFOA and PFHxS residues were non-detects, so no comparisons were made between regions. Geometric mean PFOS for both W (462 ng g^{-1}, ww) and PS (203 ng g^{-1}, ww) were significantly higher than the residues for coastal otters (45.3 ng g^{-1}, ww). Otters from the Willamette River near Albany, Oregon had the highest PFOS residues, with concentrations ranging from 279-994 ng g^{-1} (ww). Mean FOSA residues from PS (43.4 ng g^{-1}, ww) and W (17.0 ng g^{-1}, ww) were

Table 18. Regional butyltin concentrations (ng g^{-1}, ww) in livers of adult river otter males collected from Oregon and Washington, 1996-97 . Data from Kannan et al. 1999.

ID	Location	MBT	DBT	TBT	BTs	Region
Oregon						
RAG-029	Columbia River, Scappoose	19	50	2.7	72	LCR
RAG-001	Yaquina River, Toledo	23	36	3.6	53	OC
RAG-065	Yaquina River, Toledo	21	77	3.8	102	OC
RAG-230	Yaquina River, Toledo	<7	20	2.8	23	OC
RAG-022	Nehalem River, Mist	7.1	13	<1	20	OC
RAG-041	Nehalem River, Mist	27	30	1.5	59	OC
RAG-208	Nehalem River, Mist	<7	8.5	<1	8.5	OC
RAG-215	Humbug Creek, Vinemaple	<7	16	1.4	17	OC
RAG-026	Willamette River, Albany	30	34	2.7	67	W
RAG-027	Willamette River, Albany	29	10	1.9	41	W
RAG-028	Willamette River, Albany	13	15	2.1	30	W
RAG-030	Willamette River, Albany	18	34	1.8	54	W
RAG-058	Willamette River, Eugene	40	40	4.6	85	W
RAG-066	Willamette River, Albany	57	10	3.6	71	W
RAG-067[a]	Willamette River, Albany	15	11	1.6	28	W
RAG-075	Willamette River, Salem	18	29	3.5	51	W
Washington						
RAG-092[b]	Camano Island	1,200	490	12	1,700	PS
RAG-148	Fort Ward, Bainbridge Island	1,540	1,050	16	2,610	PS
RAG-192	Fort Ward, Bainbridge Island	40	100	2.3	142	PS
RAG-194	Fort Ward, Bainbridge Island	35	65	<1	100	PS
RAG-195	Fort Ward, Bainbridge Island	27	72	1.4	100	PS
RAG-157	Gig Harbor	290	470	35	795	PS
RAG-193	Bremerton	590	610	21	1,220	PS
RAG-202	Puyallup River, Tacoma	24	100	2.7	127	PS
RAG-213	Silverdale	20	55	5.3	82	PS
RAG-237	Silverdale	38	410	9.2	457	PS
RAG-214	Eglon	24	102	2	128	PS
RAG-240	Eglon	120	1,460	16	1,600	PS
RAG-218	Meadowbrook Creek, Sequim	<7	8.8	1.7	11	OP
RAG-126	Soleduck River, Forks	21	56	2	79	OP
RAG-137	Soleduck River, Forks	8.2	17	1.1	26	OP
RAG-200	Petroleum Creek	14	36	2.9	53	OP
RAG-221	Petroleum Creek	16	33	3.9	53	OP
RAG-232[b]	Petroleum Creek	<7	30	1.3	31	OP
RAG-217	Wentworth Lake, Forks	<7	8.8	1.7	11	OP
RAG-176	North River, Willapa Bay	22	17	<1	39	SW
RAG-209	Nemah River, Willapa Bay	<7	8.5	1.5	12	SW
RAG-252	Willapa River, Willapa Bay	78	72	8.4	158	SW
RAG-257	North River, Willapa Bay	14	32	1.5	48	SW
RAG-236	Hoquiam River, Grays harbor	22	82	4.7	109	SW

MBT = monobutyltin, DBT = dibutyltin, TBT = tributyltin, BTs = MBT + DBT + TBT
LCR = Lower Columbia River, OC = Oregon Coast, OP = Olympic Peninsula, SW = Southwestern Washington, W = Willamette River Basin.
[a] Yearling male. [b] Female otter

Table 19. Regional location comparisons using geometric mean butyltin concentrations (ng g^{-1}, ww) in livers of adult river otter males collected from Oregon and Washington, 1996-97. OC = Oregon Coast, OP = Olympic Peninsula, PS = Puget Sound, SW = Southwestern Washington, W = Willamette River Basin. Data from Kannan et al. 1999.

Organotin	OC	OP	PS	SW	W
n	7	7	12	5	9
MBT	**8.76 B**	**7.73 B**	**96.2 A**	**17.9 AB**	**23.7 AB**
DBT	**21.5 B**	**24.1 B**	**231 A**	**30.7 B**	**21.8 B**
TBT	1.54 A	1.91 A	5.60 A	2.14 A	2.57 A
BT Total	**29.9 B**	**32.1 B**	**367 A**	**52.2 B**	**52.0 B**

One-way ANOVA, General Linear Models Procedure, Tukey's Studentized Range Test, Alpha = 0.05. Rows sharing the same letter were not different from each other.
MBT = monobutyltin, DBT = dibutyltin, TBT = tributyltin, BT Total = MBT + DBT + TBT.
RAG-029 (LCR) was included with W otters. Otter RAG-067 from W is a yearling, RAG-092 from PS is a female and RAG-232 of OP is a female.

also significantly higher than found in the coastal otters (4.33 ng g^{-1}, ww). The highest FOSA concentrations were found in the PS, ranging from 22.3 to 71.6 ng g^{-1} (ww). No relationships were found between age or total body weight and perfluorochemical liver concentrations.

River Otter Scats

Twenty eight otter scat samples were collected throughout Oregon and Washington from 1994-99. Each scat sample consists of scats from several individual otters who frequented a given latrine site. Most of the scat samples were associated with 5 of the 7 regional locations where otter carcasses were collected. Contaminants (lipid basis) presented as geometric means by location are shown in Tables 21 and 22. Only nine scat samples were analyzed for non-ortho substituted PCBs, PCDDs and PCDFs and were associated only with LCR, WB and PS (Table 22). In general, otter scat from the LCR had the highest mean concentrations of OCs, PCBs, PCDDs and PCDFs, followed by WB and PS. Scat contaminants from OC and OP were considerably less in concentration or not detected (Table 21). Geometric mean scat concentrations of DDE, dieldrin and ΣPCBs are shown with the liver concentrations of both juvenile and

Table 20. Regional perfluorochemical concentrations (ng g^{-1}, ww) in livers of adult river otter males collected 1996-97 from Oregon and Washington. Data from Kannan et al. 2001, 2002.

ID	Location	PFOS	PFOA	FOSA	PFHxS	Region
Oregon						
RAG-001	Yaquina River, Toledo	45.3	<7.5	7.4	<4.0	OC
RAG-230	Yaquina River, Toledo	33.6	9.90	<4	<4.0	OC
RAG-208	Nehalem River, Mist	82.8	<7.5	12.9	<4.0	OC
RAG-026	Willamette River, Albany	796	<7.5	31.6	<4.0	W
RAG-027	Willamette River, Albany	660	<7.5	13.3	<4.0	W
RAG-028	Willamette River, Albany	994	18.6	44.0	67.7	W
RAG-058	Willamette River, Eugene	366	<7.5	6.60	<4.0	W
RAG-066	Willamette River, Albany	279	16.2	37.1	49.9	W
RAG-067[a]	Willamette River, Albany	861	<7.5	21.0	<4.0	W
RAG-075	Willamette River, Salem	97.1	<7.5	4.40	<4.0	W
Washington						
RAG-148	Fort Ward, Bainbridge Island	189	18.8	71.6	76.3	PS
RAG-194	Fort Ward, Bainbridge Island	141	<7.5	40.0	<4.0	PS
RAG-195	Fort Ward, Bainbridge Island	139	10.0	53.0	<4.0	PS
RAG-193	Bremerton	288	<7.5	22.3	<4.0	PS
RAG-213	Silverdale	248	<7.5	26.8	<4.0	PS
RAG-237	Silverdale	151	10.5	39.3	52.4	PS
RAG-214	Eglon	422	<7.5	52.6	<4.0	PS
RAG-240	Eglon	173	<7.5	67.1	<4.0	PS
RAG-126	Soleduck River, Forks	61.6	<7.5	<4.0	<4.0	OP
RAG-137	Soleduck River, Forks	24.6	<7.5	4.00	<4.0	OP

PFOS = Perfluorooctanesulfanate, PFOA = Perflourooctanoate, FOSA = Perflourooctanesulfonamide and FhxS = Perfluorohexanesulfonate.
OC = Oregon Coast, OP = Olympic Peninsula, PS = Puget Sound and W = Willamette River Basin.
[a] Yearling male.

adult otters on a regional basis (Table 23). Regression analysis between regional scat and liver concentrations of DDE, dieldrin and ΣPCBs (Figures 28-30) were positively correlated for both age groups, though not significantly between scat and adult liver ΣPCBs (Figure 30). The adult PS liver record appeared to be an outlier as several of the samples came from a highly contaminated site. The line for the adult samples was re-plotted without the PS data (dashed line) and improved the adjusted r^2 and significance of the line. The Congener PCB profiles of the prominent PCB congeners between regional locations expressed as a percent of the ΣPCBs analyzed showed similar

Table 21. Geometric means of OC insecticides and metabolites and PCBs (ng g^{-1}, lw) found in river otter scat samples collected from five regional locations of Oregon and Washington, 1994-99. LCR = Lower Columbia River, OC = Oregon Coast, OP = Olympic Peninsula, PS = Puget Sound and WB = Willamette River Basin.

Contaminant	LCR	WB	OC	PS	OP
n	7	3	5	11	2
HCB	212	212	7.02	56.6	70.2
OCS	1.04	NC	NC	NC	NC
DDE	5613	2976	23.6	304	170
DDD	266	7.49	NC	0.81	NC
Mirex	2.89	NC	NC	NC	NC
Oxychlordane	382	254	3.98	43.7	59.5
trans-Chlordane	20.1	NC	NC	NC	NC
cis-Chlordane	86.8	99.5	NC	2.31	20.7
trans-Nonachlor	334	553	NC	78.2	29.5
cis-Nonachlor	21.4	36.6	NC	3.66	NC
Total Chlordanes	1028	969	5.97	154	110
Heptachlor Epoxide	97.0	61.4	NC	1.93	0.45
Dieldrin	286	354	0.21	47.9	72.4
ΣSPCB	9275	6750	444	3099	366
PCB 44	2.53	NC	NC	NC	NC
PCB 49	18.2	NC	NC	NC	NC
PCB 52	33.3	NC	NC	NC	NC
PCB 60	9.89	NC	NC	7.81	NC
PCB 66/95	26.1	NC	NC	7.95	NC
PCB 70	NC	NC	NC	0.76	NC
PCB 97	NC	NC	NC	0.61	NC
PCB 99	781	449	3.17	175	39.7
PCB 101	205	0.24	NC	86.6	0.36
PCB 105	NC	NC	NC	3.91	NC
PCB 110	112	3.56	NC	33.6	NC
PCB 118	226	NC	NC	24.7	0.41
PCB 128	26.3	127	NC	72.8	0.47
PCB 138	2059	1263	78.5	241	105
PCB 141	3.80	NC	NC	7.80	NC
PCB 146	50.8	6.30	NC	35.8	0.47
PCB 149	96.3	3.25	NC	77.6	0.35
PCB 151	NC	NC	NC	9.87	NC
PCB 153	1949	2219	95.7	637	126
PCB 158	NC	NC	NC	4.48	NC
PCB 170/190	470	257	81.0	143	18.8
PCB 171	91.4	3.15	NC	19.9	NC

Table 21. Continued.

Contaminant	LCR	WB	OC	PS	OP
PCB 172	3.87	1.80	NC	4.19	NC
PCB 174	1.26	2.08	NC	8.79	NC
PCB 177	59.1	2.47	NC	16.4	NC
PCB 178	NC	2.53	NC	16.5	NC
PCB 179	NC	NC	NC	15.7	NC
PCB 180	1070	501	117	291	35.8
PCB 182/187	260	119	NC	121	8.31
PCB 183	130	63.8	NC	30.8	0.31
PCB 194	152	143	4.92	52.0	0.28
PCB 195	15.6	2.41	NC	9.23	0.18
PCB 200	5.02	NC	NC	NC	NC
PCB 201	133	70.6	NC	73.1	NC
PCB 203	85.8	50.1	NC	23.8	0.23
PCB 206	17.6	2.41	0.77	44.9	0.32

Geometric means could not be calculated for 1,2,4,5 -TCB, 1,2,34-TCB, QCB, photo-Mirex, α-HCH, β-HCH, γ-HCH, DDT, PCBs 31/28, 42, 64, 74, 87 as there were greater than 50% non-detections for each regional location.
NC = not calculated for as there were greater than 50% non-detections.

patterns with slight regional differences (Figure 31). In general, PCBs 138 and 153 represented the largest percent contribution on an almost equivalent basis, followed by PCBs 180, 99, and 170/190. However, the scat pool from Albany, OR, showed a very different profile from the other locations with PCB 153 contributing 67% of the congeners analyzed for that location. Regional PCB congener profiles of scats were similar to regional liver PCB profiles of juvenile and adult otters shown in Figures 32 and 33.

Contaminant Relationships to Morphometric Measures

Regression equations showing significant relationships between contaminants analyzed and morphometric measures of juvenile and adult male otters are shown in Tables 24 and 25. Though the equations were significant, the r^2 values were all below 50%, with most below 10%. Multiple regression

Table 22. Geometric means of non-ortho PCBs, dioxins and furans (ng kg^{-1}, lw) found in river otter scat samples collected from 3 regional locations of Oregon and Washington, 1994-99. LCR = Lower Columbia River, PS = Puget Sound and WB = Willamette River Basin.

Contaminant	LCR	WB	PS
n	3	3	3
PCB126	137	6.65	
PCB169	8.69	10.3	
2378-TCDD	2.09	40.0	19.9
Total TCDD	2.09	82.3	27.7
Total PCDD		1.98	
123478-H6CDD	2.04	27.0	16.7
123678-H6CDD	260	58.0	44.5
123789-H6CDD	2.97	40.2	1.92
Total H6CDD	551	271	157
1234678-H7CDD	3901	443	429
Total H7CDD	5715	688	687
OCDD	7695	4854	4477
2378-TCDF	467	157	332
Total TCDF	739	269	458
12378-PCDF	2.55	2.72	2.42
12478-PCDF	175	2.59	84.7
PCDF Total	449	172	187
123478-H6CDF	103	47.4	2.75
234678-H6CDF	78.4	2.10	20.3
123678-H6CDF	40.9	31.0	1.92
123789-H6CDF	24.9	21.3	11.3
Total H6CDF	1067	249	157
1234678-H7CDF	618	161	97.9
Total H7CDF	1314	410	264
OCDF	933	215	139

Geometric means could not be calculated for co-planar PCBs 77 and 81 and 1,2,3,7,8-PCDD as there were greater than 50% non-detections for each regional location

Table 23. River otter geometric mean liver (ww) and scat (lw) concentrations (ng g^{-1}) of ΣPCBs, DDE and dieldrin by regional locations.

Contaminant	n	ΣPCBs	DDE	Dieldrin
LCR				
Scat	7	9275	5613	286
Juvenile	7	245	53.4	3.96
Adult	14	509	145	6.51
WB				
Scat	3	6750	2976	354
Juvenile	8	217	57.1	7.40
Adult	19	183	71.6	8.73
OC				
Scat	5	444	23.6	0.21
Juvenile	8	66.7	12.9	1.39
Adult	19	88.1	13.4	1.46
PS				
Scat	11	3099	304	47.9
Juvenile	12	136	18.9	2.98
Adult	12	585	20.2	4.58
OP				
Scat	2	366	170	72.4
Juvenile	7	32.1	11.8	1.20
Adult	18	90.4	31.2	1.96

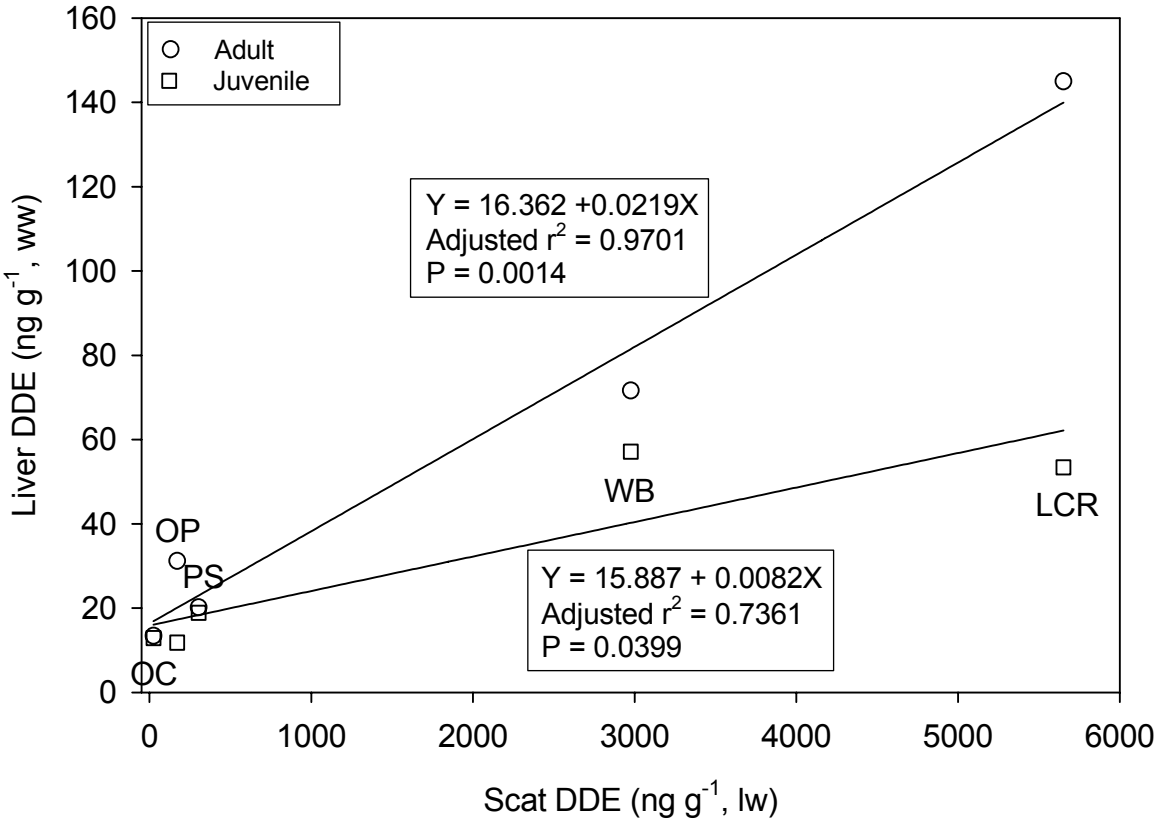


Figure 28. Relationship between river otter liver and scat DDE means from Oregon and Washington regional locations, 1994-99.

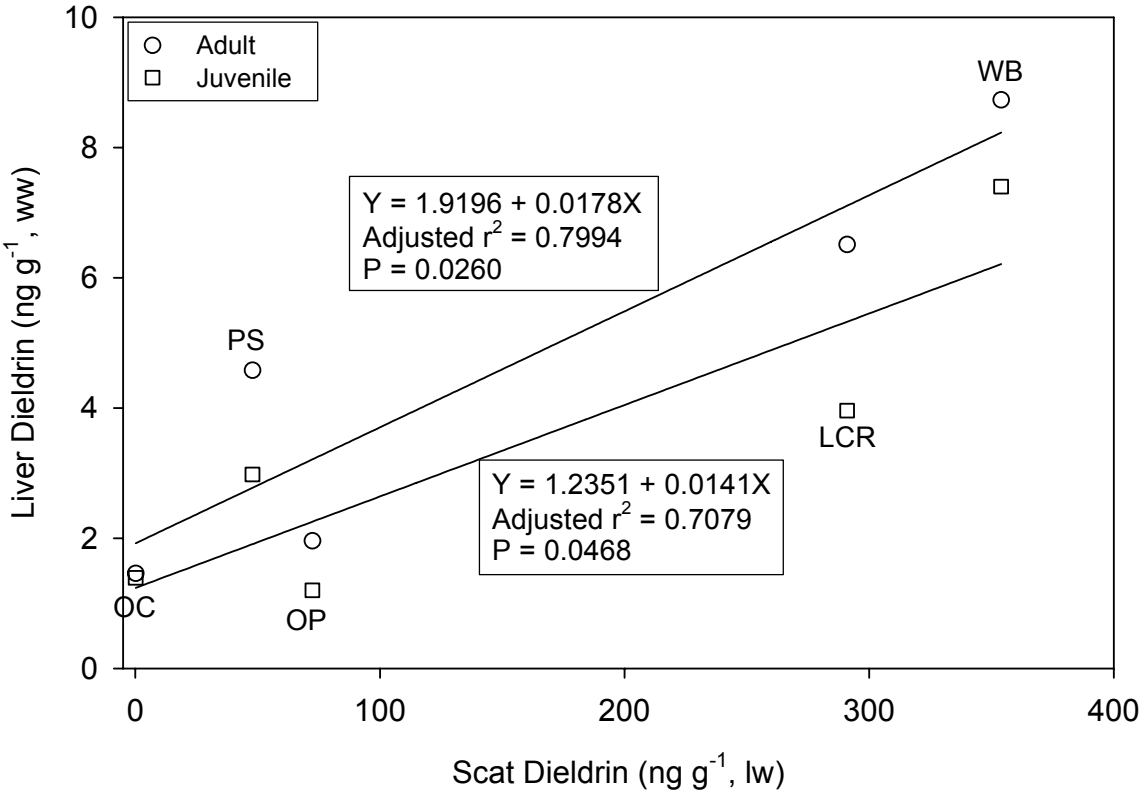


Figure 29. Relationship between river otter liver and scat dieldrin means from Oregon and Washington regional locations, 1994-99.

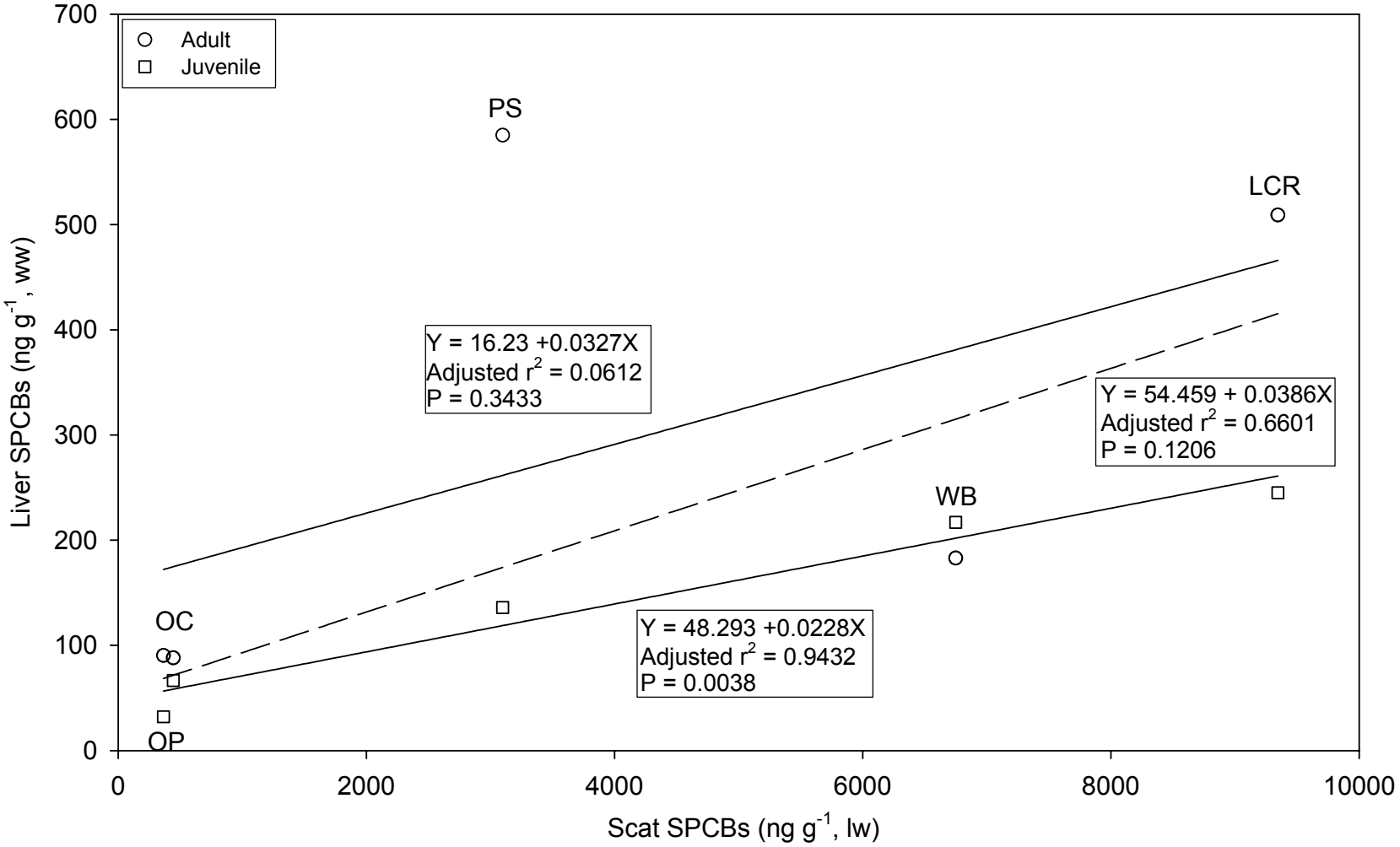


Figure 30. Relationship between river otter liver and scat sum of PCB (SPCB) means from Oregon and Washington regional locations, 1994-96. Dashed line represents adults with PS removed (see text).

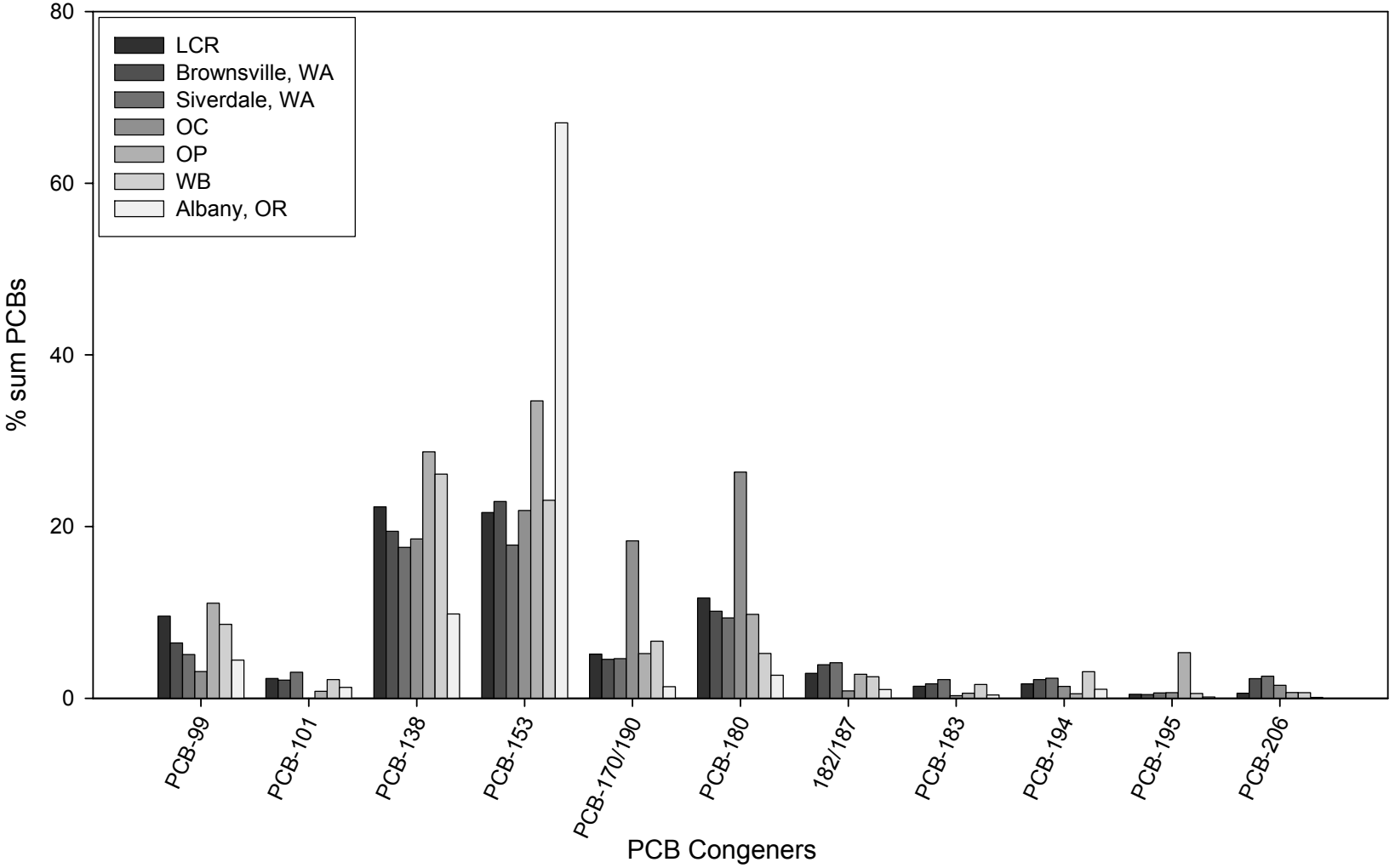


Figure 31. PCB congener profile of river otter scats as a percent of the sum of PCBs analyzed from Oregon and Washington regional locations, 1994-99.

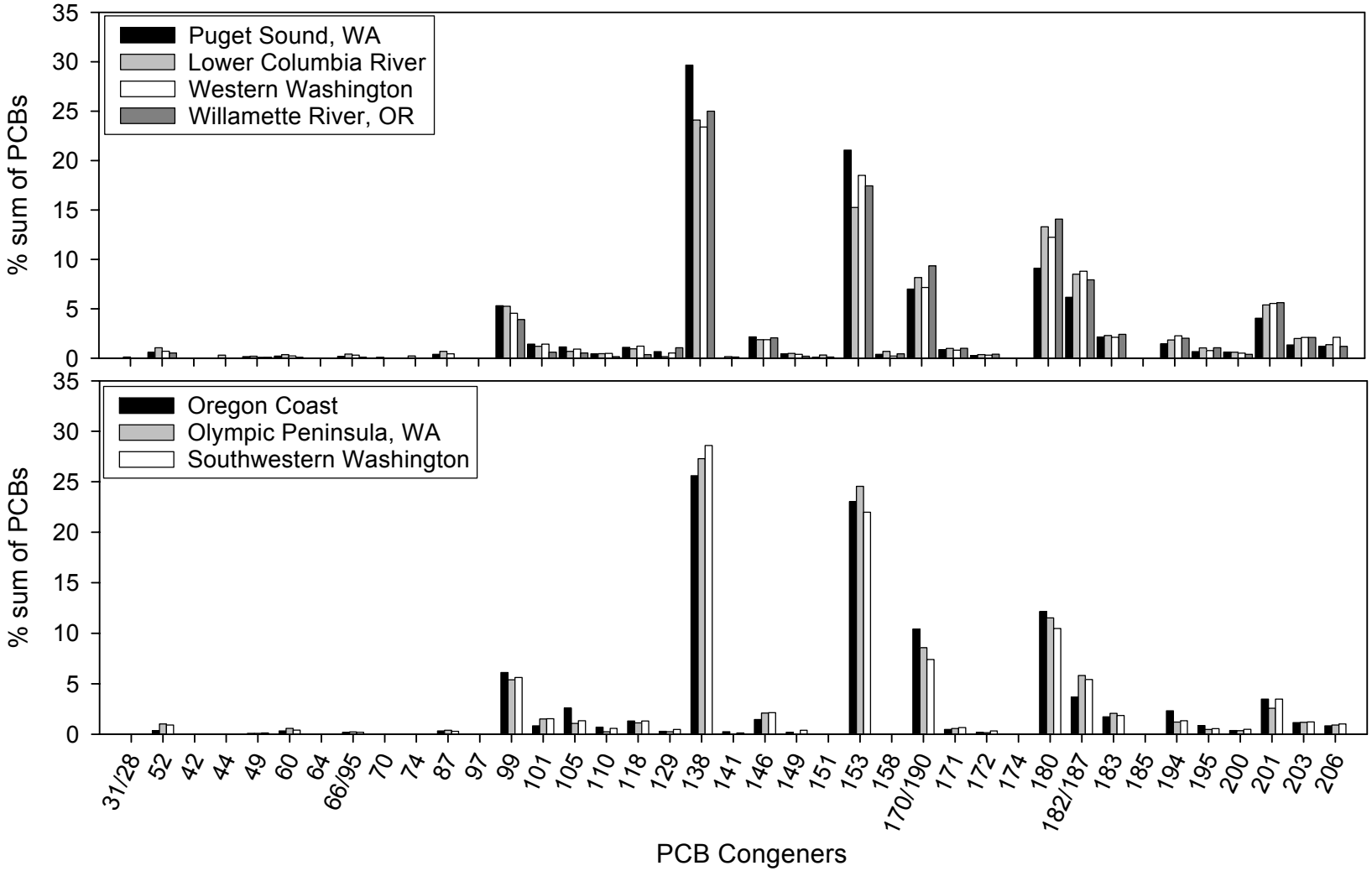


Figure 32. PCB congener profile in livers of juvenile river otter males as a percent of the sum of PCBs analyzed from Oregon and Washington regional locations, 1994-99.

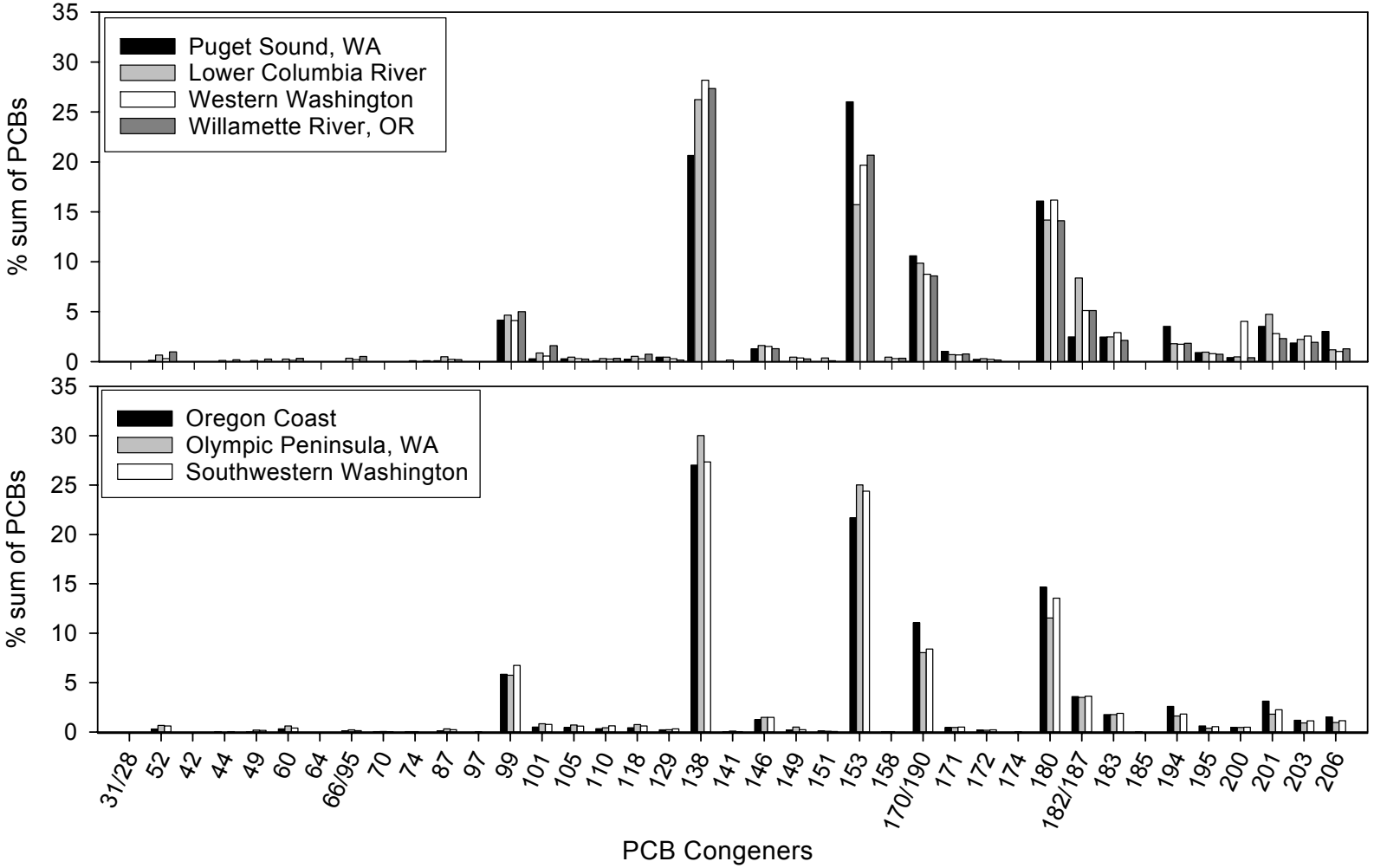


Figure 33. PCB congener profile in livers of adult river otter males as a percent of the sum of PCBs analyzed from Oregon and Washington regional locations, 1994-99.

results eliminated many of the significant relationships of earlier regression results for both juvenile and adult males (Tables 24 and 25). However several significant relationships remained. Mercury was inversely related to liver and kidney mass of juvenile male otters, and inversely related to liver, spleen, pancreas and adrenal mass of adult male otters (Figure 34).

Hexachlorobenzene was inversely related to liver, kidney and thymus mass for juveniles and thymus mass for adults (Figures 35 and 36). Thymus mass was also inversely related to HCB, *trans*-nonachlor, oxychlordane, ΣPCBs, PCB-138, PCB-153, PCB-169 and PCB-180 in juvenile males (Figure 36). Other significant relationships between contaminants and morphological measures were positive (Figure 37). A multiple regression analysis using age as a second variable showed significant inverse relationships with thymus and several PCBs (Table 26). The PCBs were arranged based on their structural similarities and the location of vicinal hydrogens (Figure 38). Congener class A (having no vicinal hydrogens) had the largest number of PCB congeners with significant inverse relationships to thymus size.

Contaminant Relationships with Male Reproductive Organs

Simple regression analyses did not reveal significant relationships between contaminant concentration and reproductive organ size in juvenile male otters. Differences in age and physical maturity of the juvenile males (as juvenile dates of birth were unknown and trapping occurred over a 4 month period) were thought to be possibly confounding any potential change in reproductive organ size with increasing contaminant concentrations. A multiple regression model using location as a random effect and adjusting for juvenile male physical maturity (using body length as an index of maturity), revealed significant inverse relationships between reproductive organ size (testes mass, gonad mass baculum length, baculum mass and prostate mass) and increasing PCB concentrations (Table 27). Figure 39 shows a three dimensional graph using baculum and ΣPCBs as the contaminant of concern. The response between the

Table 24. Regression equations showing the relationship between $\log_{10}$ total Hg, OC insecticides and metabolites, PCBs, PCDDs and PCDFs with organ mass of juvenile male river otters collected from Oregon and Washington, 1994-99. A multiple regression was also conducted using a juvenile otter Length Index as a second variable.

	Regression				Multiple Regression		
Contaminant	Equation	r^2	P	Slope	r^2	P Total Length	P Contaminant
Liver Mass (g) n = 60							
Hg	Y = 477 - 123X	0.098	0.0077	-	0.4266	<0.0001	**0.0062**
HCB	Y = 446 - 87.9X	0.080	0.0146	-	0.4495	<0.0001	**0.0017**
H7CDF	Y = 387 - 19.2X	0.077	0.0308	-	0.3471	<0.0001	0.9197
PCB TEQs	Y = 360 + 13.0X	0.049	0.0481	+	0.3481	<0.0001	0.7607
Kidney Mass (g) n = 60							
Hg	Y = 98.7 - 19.7X	0.054	0.0372	-	0.4234	<0.0001	**0.0439**
HCB	Y = 97.8 -19.8X	0.109	0.0048	-	0.5142	<0.0001	**0.0002**
H7CDD	Y = 77.1 + 6.58X	0.070	0.0216	+	0.3939	<0.0001	0.2793
H7CDF	Y = 84.3 + 4.58X	0.093	0.0092	+	0.3829	<0.0001	0.7065
Thymus Mass (g) n = 60							
HCB	Y = 16.7 - 7.42X	0.127	0.0024	-	0.2405	0.0032	**0.0009**
trans-Nonachlor	Y = 12.3 - 3.01X	0.072	0.0187	-	0.2196	0.0008	**0.0021**
Oxychlordane	Y = 15.8 - 5.94X	0.098	0.0073	-	0.2601	0.0005	**0.0004**
Sum PCBs	Y = 18.8 -3.65X	0.056	0.0341	-	0.2167	0.0006	**0.0024**
PCB-138	Y = 16.7 - 3.68X	0.059	0.0309	-	0.2214	0.0005	**0.0020**
PCB-153	Y = 17.4 - 4.62X	0.094	0.0084	-	0.2565	0.0004	**0.0005**
PCB-180	Y = 15.5 - 3.92X	0.078	0.0153	-	0.2306	0.0007	**0.0014**
PCB-169	Y = 14.8 - 5.06X	0.127	0.0026	-	0.2745	0.0007	**0.0002**
Spleen Mass (g) n = 60							
Hg	Y = 47.6 - 15.1X	0.050	0.0446	-	0.2732	<0.0001	0.0668
PCB-126	Y = 30.9 + 6.02X	0.056	0.0369	+	0.2426	0.0002	0.2816
H6CDD	Y = 35.5 + 5.09X	0.130	0.0025	+	0.2998	0.0003	**0.0179**
H7CDD	Y = 28.0 + 8.12X	0.193	0.0002	+	0.3319	0.0007	**0.0041**

Table 24. Continued.

	Regression				Multiple Regression		
Contaminant	Equation	r^2	P	Slope	r^2	P Total Length	P Contaminant
Spleen Mass (g) n = 60							
OCDD	Y = 24.6 + 7.97X	0.153	0.0011	+	0.2960	0.0008	**0.0214**
H7CDF	Y = 36.9 + 3.96X	0.114	0.0045	+	0.2471	0.0014	0.2216
PCB TEQs	Y = 31.9 + 2.36X	0.068	0.0236	+	0.2306	0.0002	0.6025
Furan TEQs	Y = 33.6 + 1.74X	0.232	<0.0001	+	0.2316	0.0004	0.5572
Adrenal Mass (g) n = 60							
Dieldrin	Y = 0.873 + 0.128X	0.053	0.0402	+	0.1462	0.0076	0.2529
Sum PCBs	Y = 0.679 + 0.122X	0.057	0.0343	+	0.1608	0.0057	0.1294
PCB-180	Y = 0.798 +0.121X	0.065	0.0261	+	0.1726	0.0044	**0.0776**
H6CDD	Y = 0.905 + 0.054X	0.061	0.0306	+	0.1626	0.0070	**0.0070**
H7CDD	Y = 0.793 + 0.115X	0.181	0.0004	+	0.2440	0.0197	**0.0039**
OCDD	Y = 0.730 + 0.123X	0.171	0.0005	+	0.2231	0.0250	**0.0091**
H7CDF	Y = 0.920 + 0.055X	0.103	0.0067	+	0.1628	0.0351	0.1058
Furan TEQs	Y = 0.865 + 0.029X	0.312	<0.0001	+	0.1521	0.0179	0.1730

Table 25. Regression equations showing the relationship between log_{10} total Hg, OC insecticides and metabolites, PCBs, PCDDs and PCDFs with organ masses of adult male river otters collected from Oregon and Washington, 1994-99. A multiple regression was also conducted using the otters total body length as a second variable.

	Regression				Multiple Regression		
Contaminant	Equation	r^2	P	Slope	r^2	P Total Length	P Contaminant
Liver Mass (g) n = 93							
Hg	Y = 608 - 113X	0.095	**0.0014**	-	0.3382	<0.0001	**0.0003**
trans-Nonachlor	Y = 482 + 39.3X	0.041	**0.0268**	+	0.2432	<0.0001	0.3460
Dieldrin	Y = 476 + 45.0X	0.040	**0.0280**	+	0.2412	<0.0001	0.4196
Sum PCBs	Y = 368 + 59.3X	0.057	**0.0107**	+	0.2373	<0.0001	0.6591
PCB-138	Y = 395 + 63.4X	0.068	**0.0060**	+	0.2391	<0.0001	0.5200
PCB-153	Y = 419 + 52.4X	0.041	**0.0261**	+	0.2358	<0.0001	0.8927
PCB-180	Y = 426 + 54.7X	0.054	**0.0126**	+	0.2373	<0.0001	0.6522
H6CDD	Y = 498 + 23.6X	0.064	**0.0073**	+	0.2639	<0.0001	0.0663
H7CDD	Y = 479 + 23.9X	0.042	**0.0261**	+	0.2426	<0.0001	0.3654
OCDD	Y = 466 + 25.8X	0.035	**0.0368**	+	0.2365	<0.0001	0.7418
H7CDF	Y = 503 + 18.1X	0.052	**0.0146**	+	0.2605	<0.0001	0.0851
PCB TEQs	Y = 485 + 6.13X	0.064	**0.0076**	+	0.2440	<0.0001	0.3208
Dioxin TEQs	Y = 486 + 8.93X	0.044	**0.0221**	+	0.2392	<0.0001	0.5166
Furan TEQs	Y = 481 + 11.6X	0.070	**0.0053**	+	0.2638	<0.0001	0.0665
Total TEQs	Y = 481 + 3.31X	0.073	**0.0045**	+	0.2451	<0.0001	0.2892
Kidney Mass (g) n = 93							
trans-Nonachlor	Y = 104 + 7.32X	0.047	**0.0195**	+	0.4115	<0.0001	0.3937
Sum PCBs	Y = 67.8 + 17.7X	0.182	**<0.0001**	+	0.4438	<0.0001	**0.0163**
PCB-138	Y = 78.5 + 17.4X	0.178	**<0.0001**	+	0.4390	<0.0001	**0.0253**
PCB-153	Y = 79.3 + 17.9X	0.184	**<0.0001**	+	0.4481	<0.0001	**0.0110**
PCB-180	Y = 84.9 + 16.5X	0.178	**<0.0001**	+	0.4454	<0.0001	**0.0140**
H6CDD	Y = 107 + 3.28X	0.036	**0.0363**	+	0.4176	<0.0001	0.1980
H7CDD	Y = 103 + 4.58X	0.051	**0.0155**	+	0.4104	<0.0001	0.4526

Table 25. Continued.

	Regression				Multiple Regression		
Contaminant	Equation	r^2	P	Slope	r^2	P Total Length	P Contaminant
Kidney Mass (g) n = 93							
OCDD	Y = 98.7 +6.36X	0.079	**0.0033**	+	0.4115	<0.0001	0.3936
H7CDF	Y = 108 + 2.68X	0.033	**0.0421**	+	0.4134	<0.0001	0.3147
PCB TEQs	Y = 105 +0.91X	0.042	**0.0258**	+	0.4193	<0.0001	0.1659
Dioxin TEQs	Y = 105 + 1.47X	0.037	**0.0332**	+	0.4139	<0.0001	0.2947
Furan TEQs	Y = 104 + 1.96X	0.064	**0.0075**	+	0.4155	<0.0001	0.2483
Total TEQs	Y = 104 + 0.52X	0.056	**0.0113**	+	0.4231	<0.0001	0.1135
Thyroid Mass (g) n = 93							
HCB	Y = 0.71 + 0.24X	0.062	**0.0085**	+	0.1605	0.0005	**0.0266**
trans-Nonachlor	Y = 0.85 + 0.11X	0.038	**0.0322**	+	0.1234	0.0008	0.3057
PCB-153	Y = 0.68 + 0.14X	0.037	**0.0332**	+	0.1150	0.0011	0.6645
Thymus Mass (g) n = 93							
HCB	Y = 8.09 - 2.01X	0.034	**0.0404**	-	0.0285	0.4863	**0.0386**
H7CDD	Y = 7.03 - 0.67X	0.033	**0.0419**	-	0.0331	0.2956	**0.0302**
Spleen Mass (g) n = 93							
Hg	Y = 56.1 - 8.96X	0.034	**0.0402**	-	0.0906	0.0122	**0.0357**
H7CDD	Y = 44.5 + 3.40X	0.060	**0.0092**	+	0.0836	0.0459	0.0536
OCDD	Y = 42.0 + 4.08X	0.066	**0.0065**	+	0.0830	0.0673	0.0533
H7CDF	Y = 47.9 + 2.71X	0.082	**0.0027**	+	0.1071	0.0261	**0.0139**
Pancreas Mass (g) n = 93							
Hg	Y = 44.5 - 6.72X	0.059	**0.0101**	-	0.1252	0.0059	**0.0090**
H6CDD	Y = 37.9 + 1.36X	0.034	**0.0404**	+	0.0793	0.0139	0.1345
PCB TEQs	Y = 37.0 + 0.41X	0.050	**0.0164**	+	0.0605	0.0107	0.5098
Dioxin TEQs	Y = 37.0 + 0.68X	0.048	**0.0186**	+	0.0751	0.1760	**0.0155**
Furan TEQs	Y = 36.9 + 0.67X	0.040	**0.0288**	+	0.0712	0.0177	0.2273
Total TEQs	Y = 36.8 + 0.22X	0.058	**0.0107**	+	0.0620	0.0179	0.4493

Table 25. Continued.

	Regression				Multiple Regression		
Contaminant	Equation	r^2	P	Slope	r^2	P Total Length	P Contaminant
Adrenal Mass (g) n = 93							
Hg	Y = 1.26 - 0.23X	0.051	**0.0153**	-	0.1009	0.0145	**0.0159**
Sum PCBs	Y = 0.61 +0.19X	0.085	**0.0023**	+	0.0977	0.1171	**0.0191**
PCB-138	Y = 0.75 + 0.17X	0.069	**0.0058**	+	0.0839	0.1049	**0.0420**
PCB-153	Y = 0.78 + 0.16X	0.059	**0.0098**	+	0.0766	0.0865	0.0642
PCB-180	Y = 0.75 + 0.21X	0.124	**0.0003**	+	0.1370	0.1539	**0.0021**
H6CDD	Y = 1.03 + 0.08X	0.113	**0.0005**	+	0.1151	0.0393	**0.0072**
H7CDD	Y = 0.96 + 0.08X	0.080	**0.0030**	+	0.1021	0.0680	**0.0149**

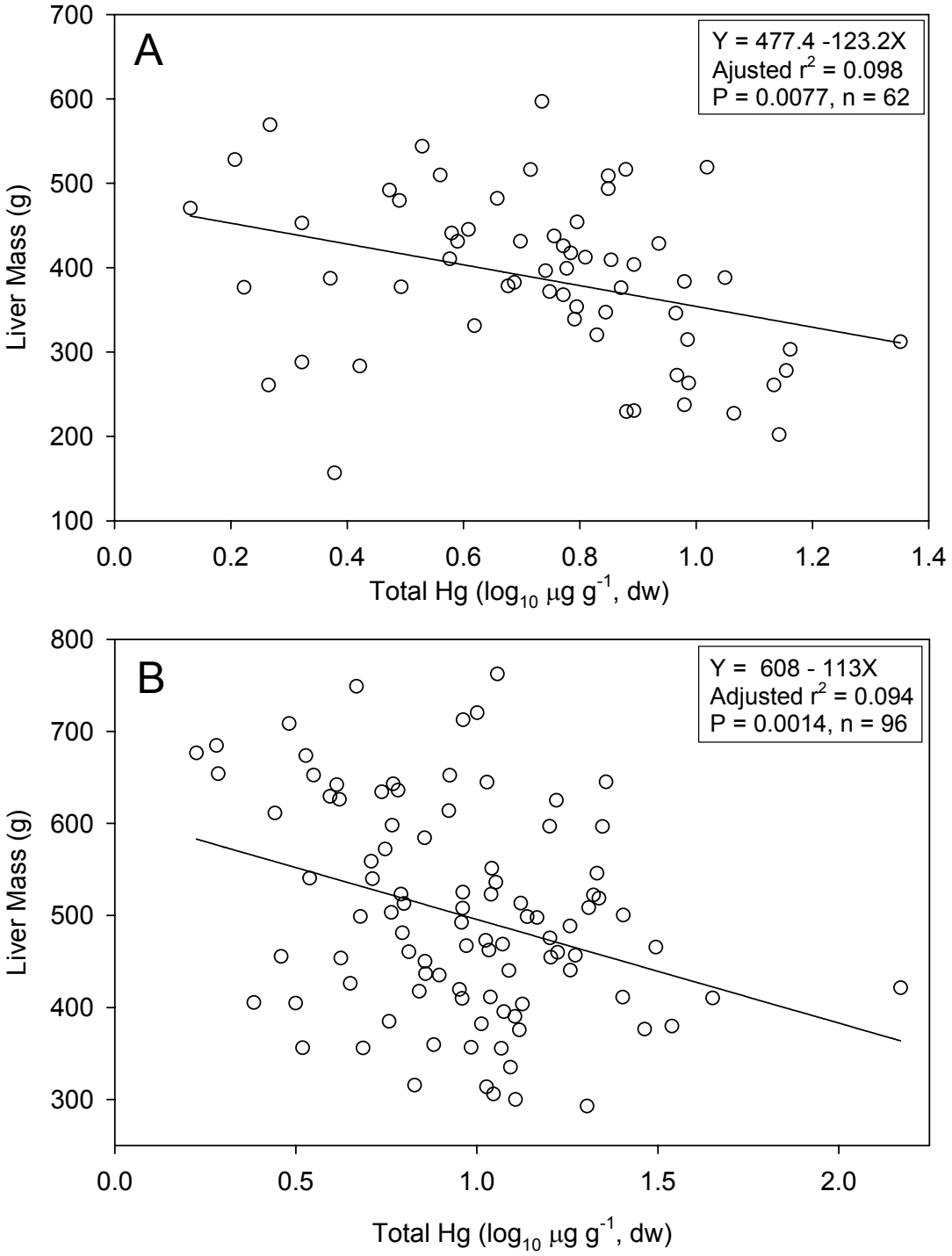


Figure 34. Relationships between liver Hg concentrations and liver mass of juvenile (A) and adult (B) river otter males collected from Oregon and Washington, 1994-99.

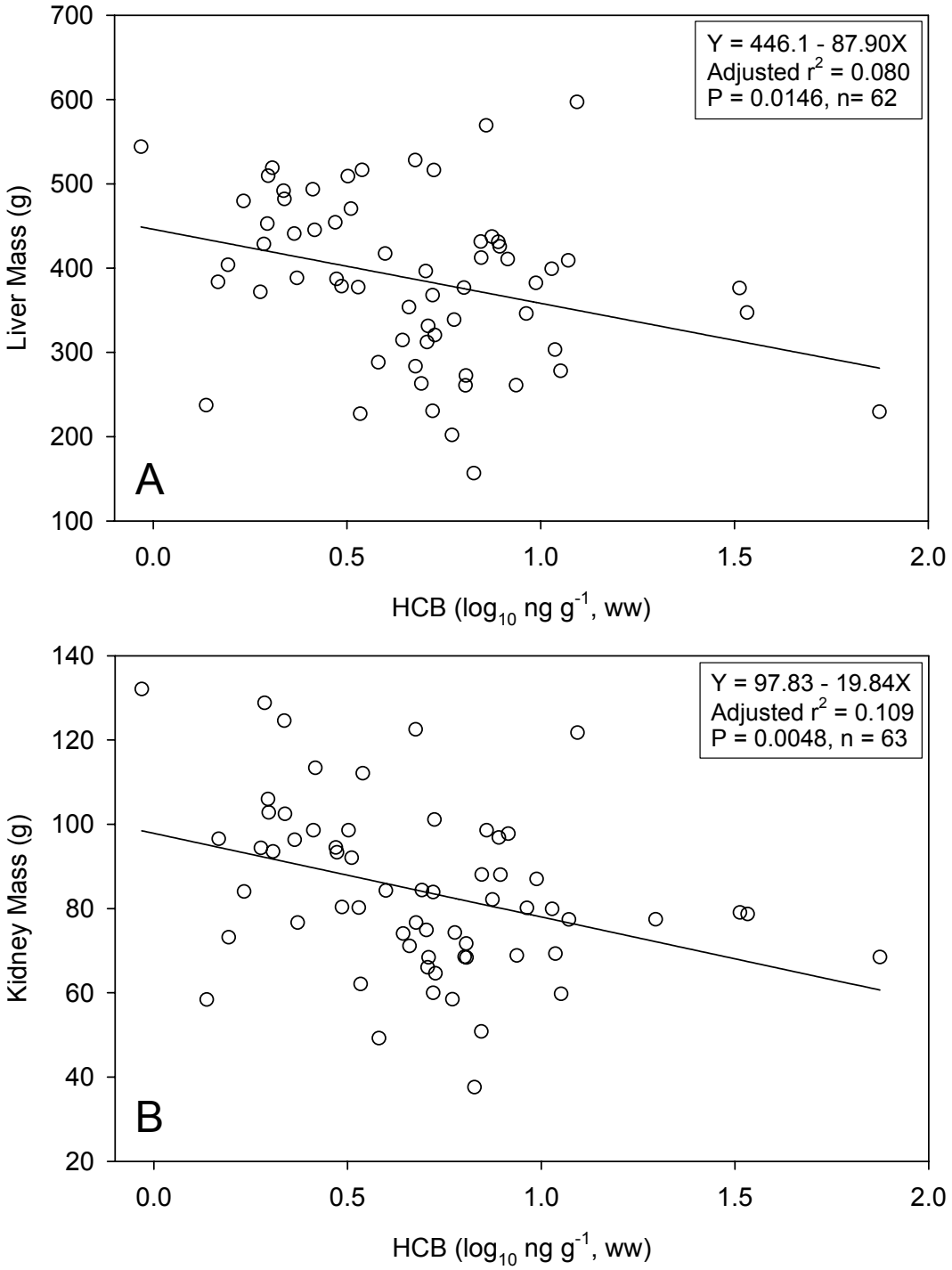


Figure 35. Relationships between liver HCB concentrations and liver mass (A) and kidney mass (B) of juvenile river otter males collected from Oregon and Washington, 1994-99.

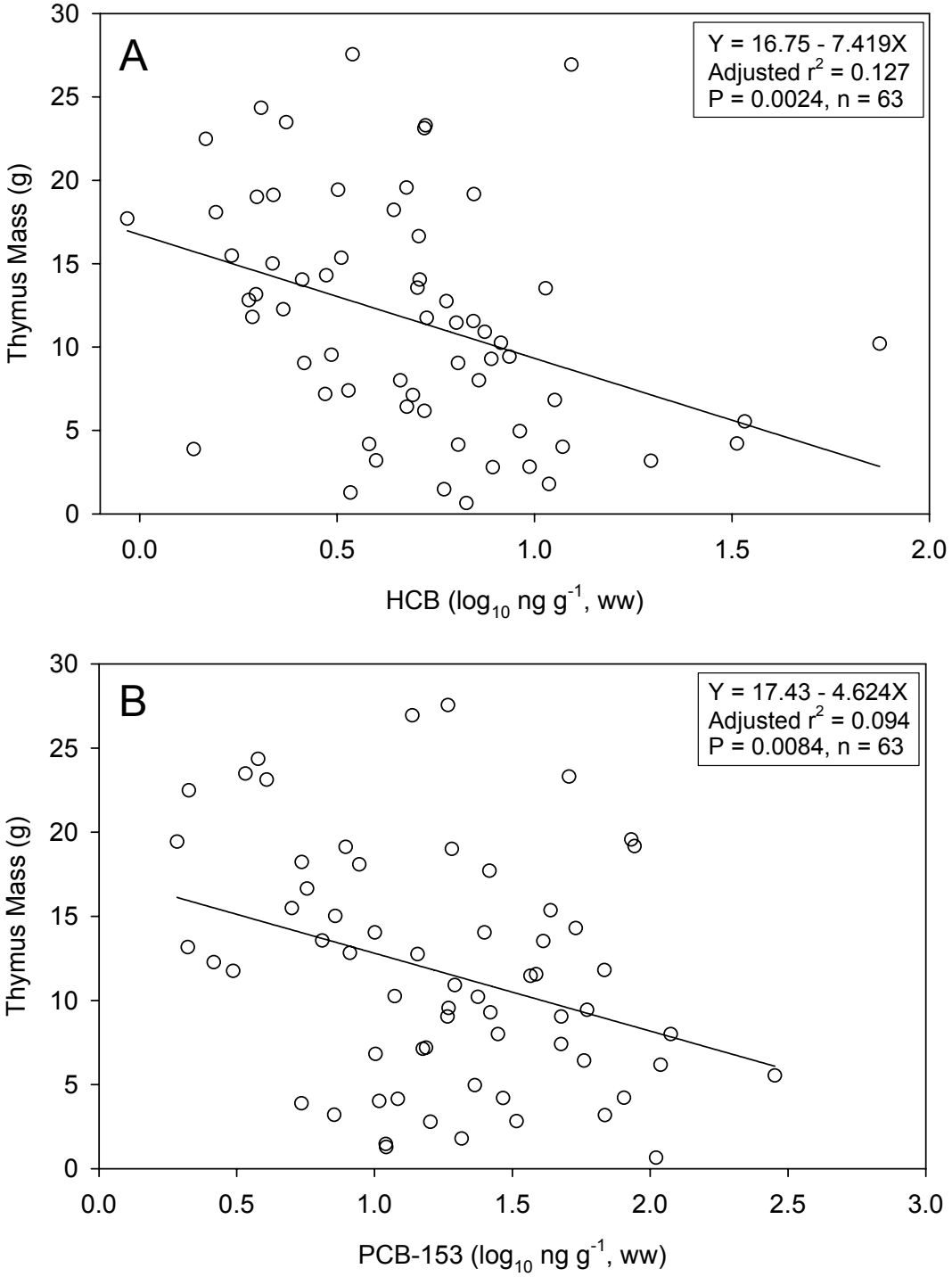


Figure 36. Relationships between liver HCB concentrations and thymus mass (A) and liver PCB 153 concentrations and thymus mass (B) of juvenile river otter males collected from Oregon and Washington, 1994-99.

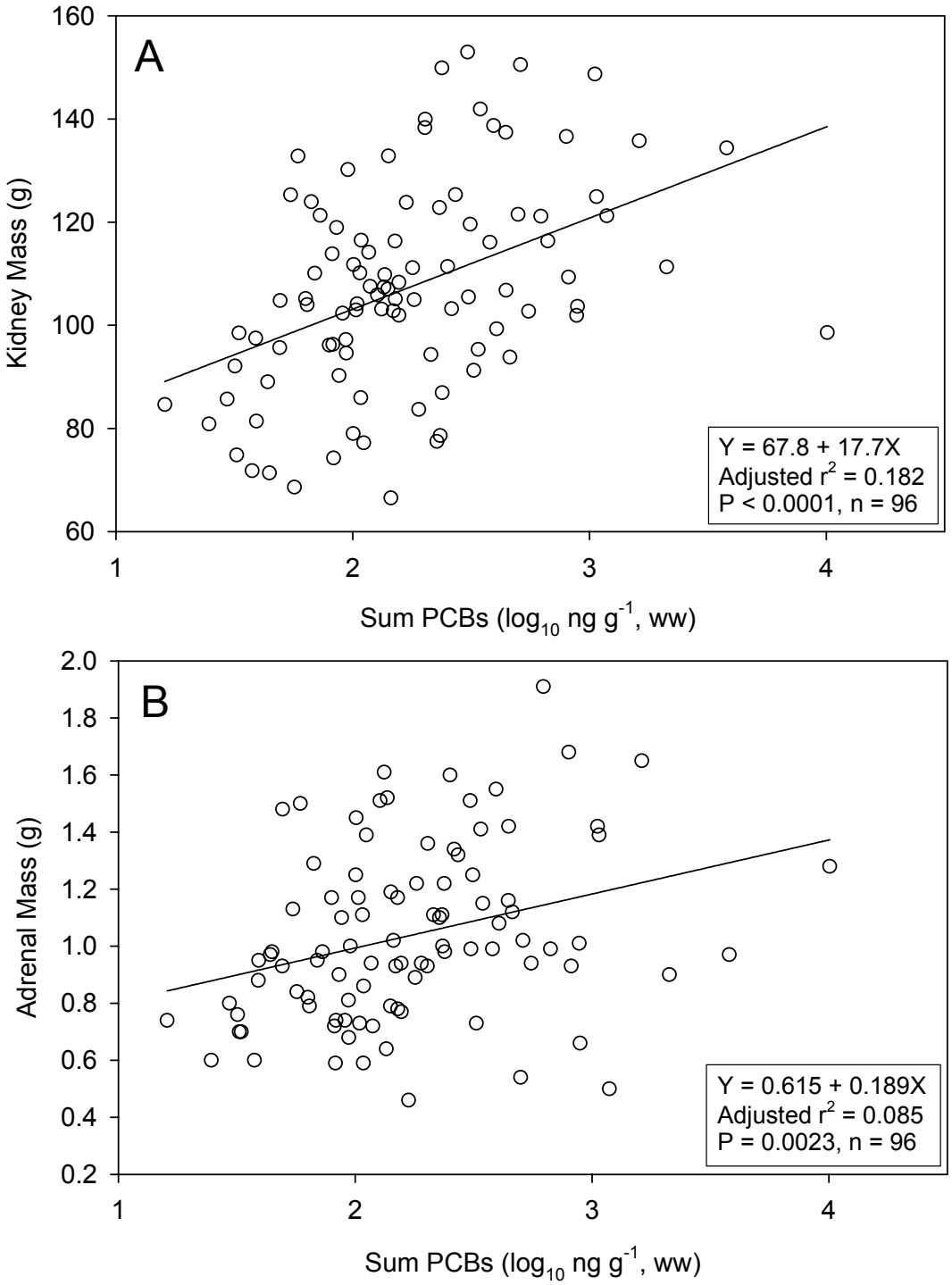


Figure 37. Relationship between liver sum of PCB concentrations and kidney mass (A) and adrenal mass (B) of adult river otter males collected from Oregon and Washington, 1994-99.

Table 26. Multiple regression showing the relationship between $\log_{10}$ PCBs with thymus mass using age as a second variable of male river otters collected from Oregon and Washington, 1994-99. PCB congeners were classed based on structural similarities: class A, no vicinal hydrogens present; class B, vicinal hydrogens in the ortho-meta configuration; class C, vicinal hydrogens in the meta-para configuration; class D, vicinal hydrogens in both the ortho-meta and meta-para configuration. Means are from logged values and percent is of SPCBs (n = 180).

Congener	*Ortho*-Substitution	Class	Mean Concentration	Percent ΣPCBs	Correlation (+ or -)	P Value
PCB 169	None	A	0.006	0.004	-	**0.0006**
PCB 146	2,2'	A	2.13	1.50	-	0.0586
PCB 153	2,2'	A	29.3	20.6	-	**0.0057**
PCB 172	2,2'	A	0.22	0.15	-	**0.0062**
PCB 180	2,2'	A	17.4	12.3	-	**0.0036**
PCB 194	2,2'	A	2.11	1.49	-	**0.0009**
PCB 183	2,2',6	A	2.59	1.82	-	**0.0316**
PCB 203	2,2',6	A	1.82	1.28	-	**0.0255**
PCB 206	2,2',6	A	1.67	1.17	-	**0.0002**
PCB 182/187	2,2',6'/2,2',6'	A	6.40	4.51	-	0.1583
PCB 201	2,2',6'	A	4.56	3.21	-	0.1085
PCB 200	2,2',6,6'	A	0.51	0.36	-	**0.0005**
PCB 126	None	B	0.009	0.006	-	0.3519
PCB 60	2	B	0.16	0.11	-	0.0553
PCB 105	2	B	0.34	0.24	-	0.0525
PCB 118	2	B	0.67	0.47	-	0.1963
PCB 99	2,2'	B	6.72	4.73	-	**0.0257**
PCB 138	2,2'	B	37.3	26.3	-	**0.0155**
PCB 158	2,6	B	0.03	0.02	-	0.4378
PCB 170/190	2,2'/2,6	B	12.1	8.54	-	**0.0117**

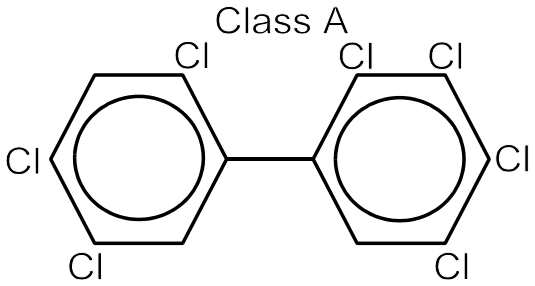
Table 26. Continued.

Congener	*Ortho*-Substitution	Class	Mean Concentration	Percent ΣPCBs	Correlation (+ or -)	P Value
PCB 171	2,2',6	B	0.64	0.45	-	**0.0263**
PCB 195	2,2',6	B	0.70	0.50	-	**0.0008**
PCB 66/95	2/2,6	B,C	0.08	0.06	-	0.3807
PCB 52	2,2'	C	0.38	0.27	-	0.9565
PCB 101	2,2'	C	0.92	0.65	-	0.4786
PCB 129	2,2'	C	0.28	0.20	-	0.0540
PCB 141	2,2'	C	0.02	0.01	-	0.7612
PCB 149	2,2',6	C	0.13	0.09	-	0.7842
PCB 151	2,2',6	C	0.03	0.02	-	0.8106
PCB 44	2,2'	D	0.01	0.01	-	0.6140
PCB 49	2,2'	D	0.04	0.03	-	0.4379
PCB 87	2,2'	D	0.10	0.07	-	**0.0189**
PCB 110	2,6	D	0.14	0.10	-	0.4483

Note: The P value for the negative age relationship with thymus mass was always <0.0001.

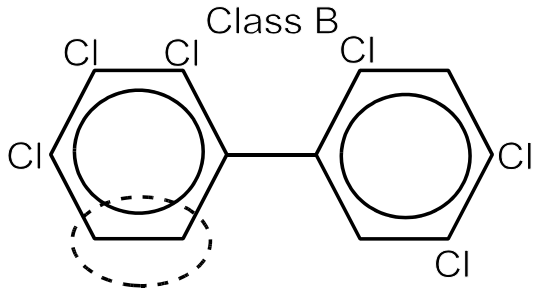
predicted reproductive organ size and the length index (i.e., an indicator of maturity) is substantial. However, accounting for juvenile age via physical size using the length index, the data reveals a subtle, but distinct negative contaminant effect. The lower and upper 95% confidence limits shown in Table 26 represent the estimated change in reproduction organ size and mass associated with a 100-fold increase in contaminant concentration after an age adjustment for juveniles using the body length index. For example, the lower and upper confidence limits for testes mass shows the potential estimated change of -47 to 4.4% for every 100-fold increase in ΣPCBs (Table 27). This interval represents a range of potential '**decrease**' in testes mass of 47% to a potential '**increase**' of 4.4%. Individual congeners significantly related to testes mass in this model also showed similar changes. The estimated change in baculum length and prostate mass was -12 to -1% and -39 to 3.0% for every 100-fold increase in ΣPCB concentration, respectively. Baculum mass was also significantly reduced for several individual congeners, though not for the ΣPCBs. DDE was also negatively related to baculum length and baculum mass, with HCB negatively related to prostate mass. DDE was only associated with the baculum in both length and mass and not with any soft tissues. The estimate column calculated by the model bisects the confidence interval range, and should viewed as such. Figures 40 through 42 show some of the inverse relationships between reproductive organ size and select contaminants.

Multiple regression analyses showed that baculum length and mass of adult male otters also was inversely related to contaminant concentrations (Table 28). Baculum length of adult males was inversely related to PCB congeners only (Figure 43). However, baculum mass was also shown to be inversely related to several OCs, especially the DDT metabolites DDD and DDE (Figure 44). Some of the chlordanes and dieldrin also showed negative correlations with baculum mass (Figure 45). These findings with adult males imply that the reduced baculum size was permanent.



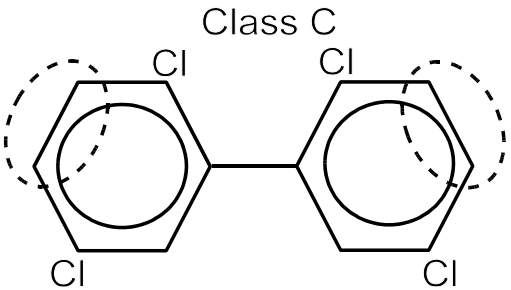
PCB 180

no vicinal hydrogens



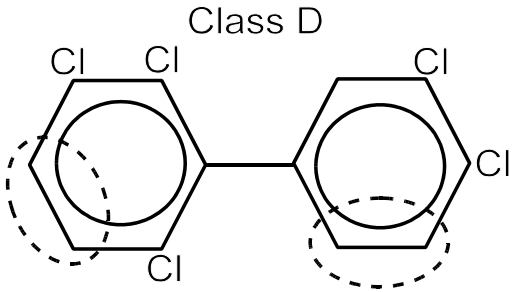
PCB 138

vicinal ortho-meta hydrogens



PCB 52

vicinal meta-para hydrogens



PCB 110

both ortho-meta and meta-para vicinal hydrogens

Figure 38. PCB congeners classed based on structural similarities, with dotted circles showing vicinal hydrogens.

Table 27. Contaminant effects on testes mass, gonad mass (testes + epididymis mass), baculum length, baculum mass, and prostate mass of juvenile male river otters using a multiple regression model with location as a random effect (i.e., accounting for otter measurements from the same location which might be correlated). The lower and upper columns represent the 95% confidence limits of the estimated change in the reproductive parameter associated with a 100-fold increase in contaminant concentration after adjusting for individual physical maturity using a body length index (juvenile total length/mean adult male total length). P is the p-value of the test that the change is actually zero, with alpha = 0.1.

Contaminant	Range (ng g^{-1}, ww)	Estimate	Lower	Upper	P
Testicle Mass (g) n = 53					
Sum PCBs	9.01 - 1857	-25.5	-46.9	4.4	0.0853
PCB 99	1nd - 60.7	-22.9	-40.1	-0.7	0.0445
PCB 138	2.30 - 458	-25.7	-46.5	3.1	0.0742
PCB 146	0.20 - 42.7	-27.7	-48.0	0.2	0.0529
PCB 153	1.92 - 284	-27.6	-48.3	1.4	0.0599
Gonad Mass (g) n = 59					
PCB 180	0.56 - 259	-32.5	-56.8	5.3	0.0821
PCB 201	0.30 - 123	-16.9	-33.4	7.7	0.1000
Baculum Length (mm) n = 56					
DDE	0.46 - 1505	-5.9	-10.6	-1.0	0.0190
Sum PCBs	9.01 - 1857	-7.0	-12.4	-1.3	0.0185
PCB-99	1nd - 60.7	-5.4	-9.6	-1.0	0.0181
PCB-101	8nd - 52.4	-5.0	-7.8	-2.1	0.0013
PCB-138	2.30 - 458	-7.3	-12.5	-1.7	0.0122
PCB-146	0.20 - 42.7	-7.5	-12.8	-2.0	0.0097
PCB-153	1.92 - 284	-7.2	-12.7	-1.3	0.0182
PCB-170/190	0.54 - 192	-5.9	-11.3	-0.2	0.0415
PCB-180	0.56 - 259	-5.6	-10.8	-0.1	0.0477
PCB-182/187	0.39 - 157	-6.2	-11.2	-0.9	0.0233
PCB-183	0.22 - 48.9	-6.7	-11.9	-1.2	0.0192
PCB-201	0.30 - 123	-6.1	-11.1	-0.9	0.0224
PCB-203	1nd - 41.6	-4.9	-9.4	-0.2	0.0435
PCB-169	1nd - 46.4	-5.0	-10.2	-0.5	0.0741
Baculum Mass (g) n = 56					
DDE	0.46 - 1505	-15.4	-30.6	3.1	0.0950
PCB-101	8nd - 52.4	-11.2	-21.6	0.5	0.0593
Prostate Mass (g) n = 51					
HCB	0.93 - 74.8	-31.2	-52.8	-0.3	0.0515
Sum PCBs	9.01 - 1857	-20.8	-39.1	3.1	0.0816
PCB-99	1nd - 60.7	-17.9	-32.5	-0.2	0.0478
PCB-153	1.92 - 284	-20.2	-39.0	4.4	0.0980
PCB-170/190	0.54 - 192	-25.0	-41.5	-3.8	0.0246
PCB-180	0.56 - 259	-22.7	-39.3	-1.6	0.0370
PCB-203	1nd - 41.6	-16.6	-32.0	2.3	0.0795
PCB-169	1nd - 46.4	-24.0	-40.0	-3.8	0.0234

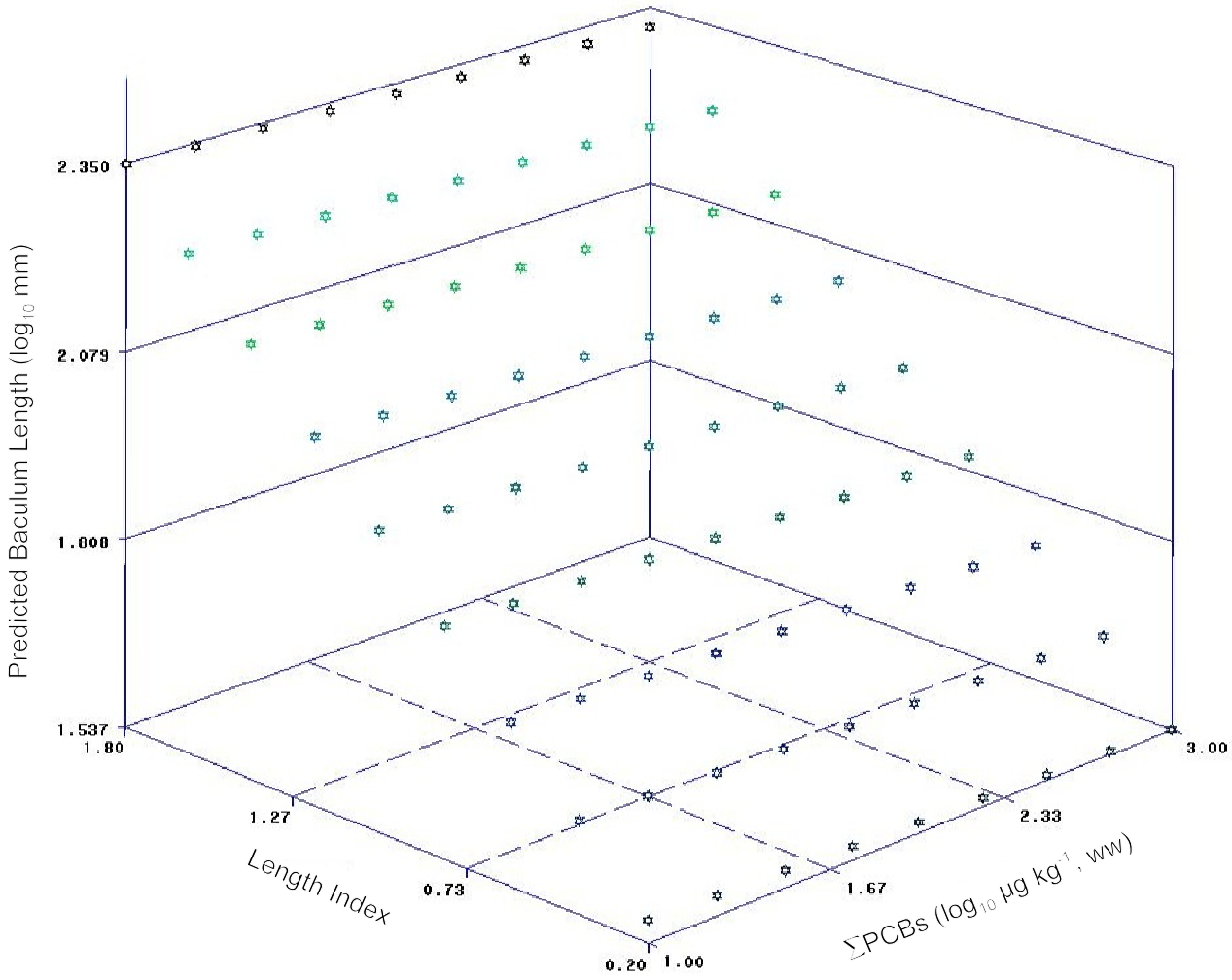


Figure 39. Three dimensional graph showing the predicted effects of increasing ΣPCB concentrations on baculum length in juvenile river otter males from Oregon and Washington, 1994-99 (Table 27). Predicted baculum length (log_{10} mm) represents the z axis, length index the y axis and ΣPCBs (log_{10} µg kg^{-1}, ww) the x axis. The multiple regression model used location as a random effect. The graph shows a small but significant negative response to increasing ΣPCB concentrations.

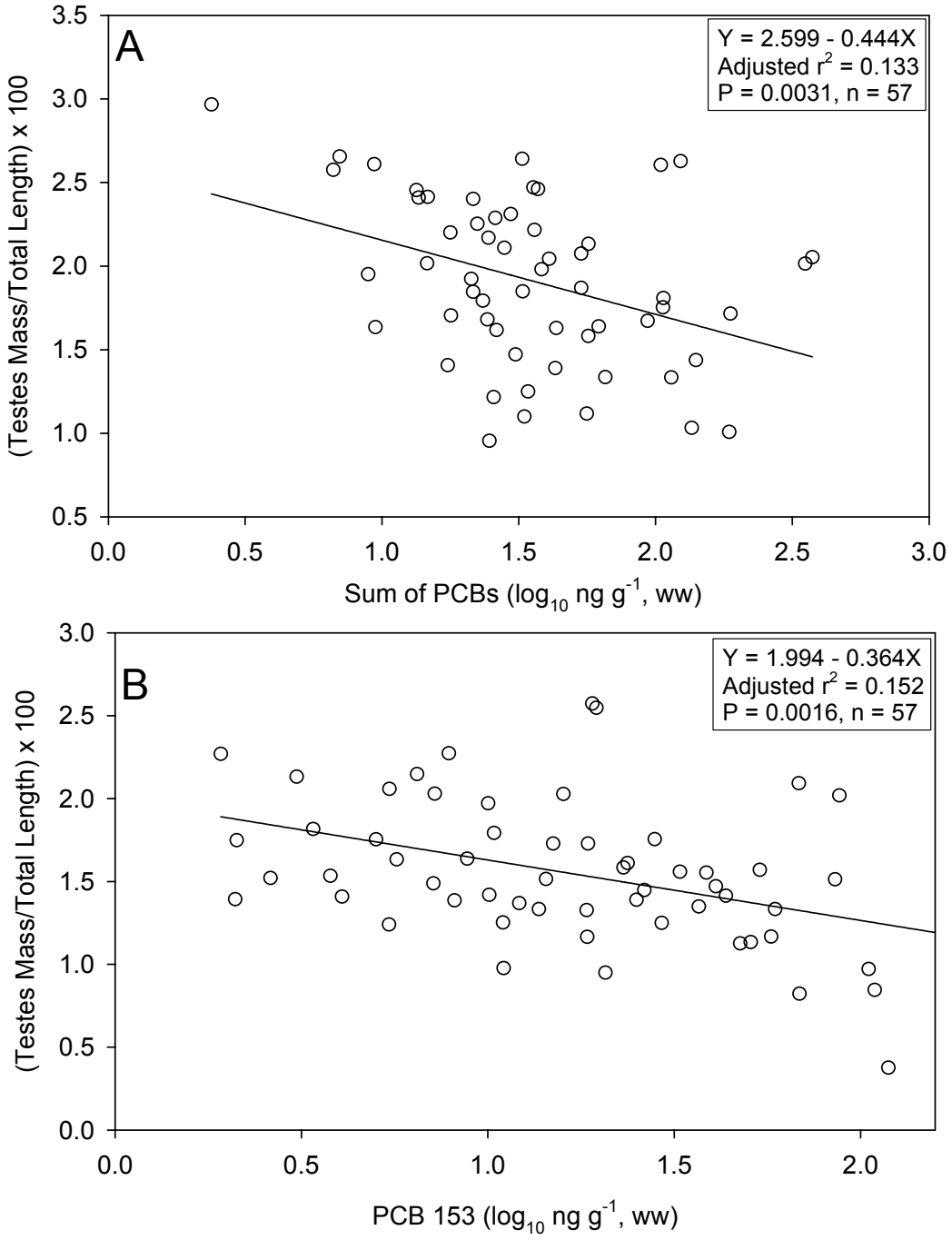


Figure 40. Relationships between (testes mass (g)/total body length (cm)) x 100 and sum of PCBs (A) and PCB 153 (B) analyzed in livers of juvenile river otter males collected from Oregon and washington, !994-99.

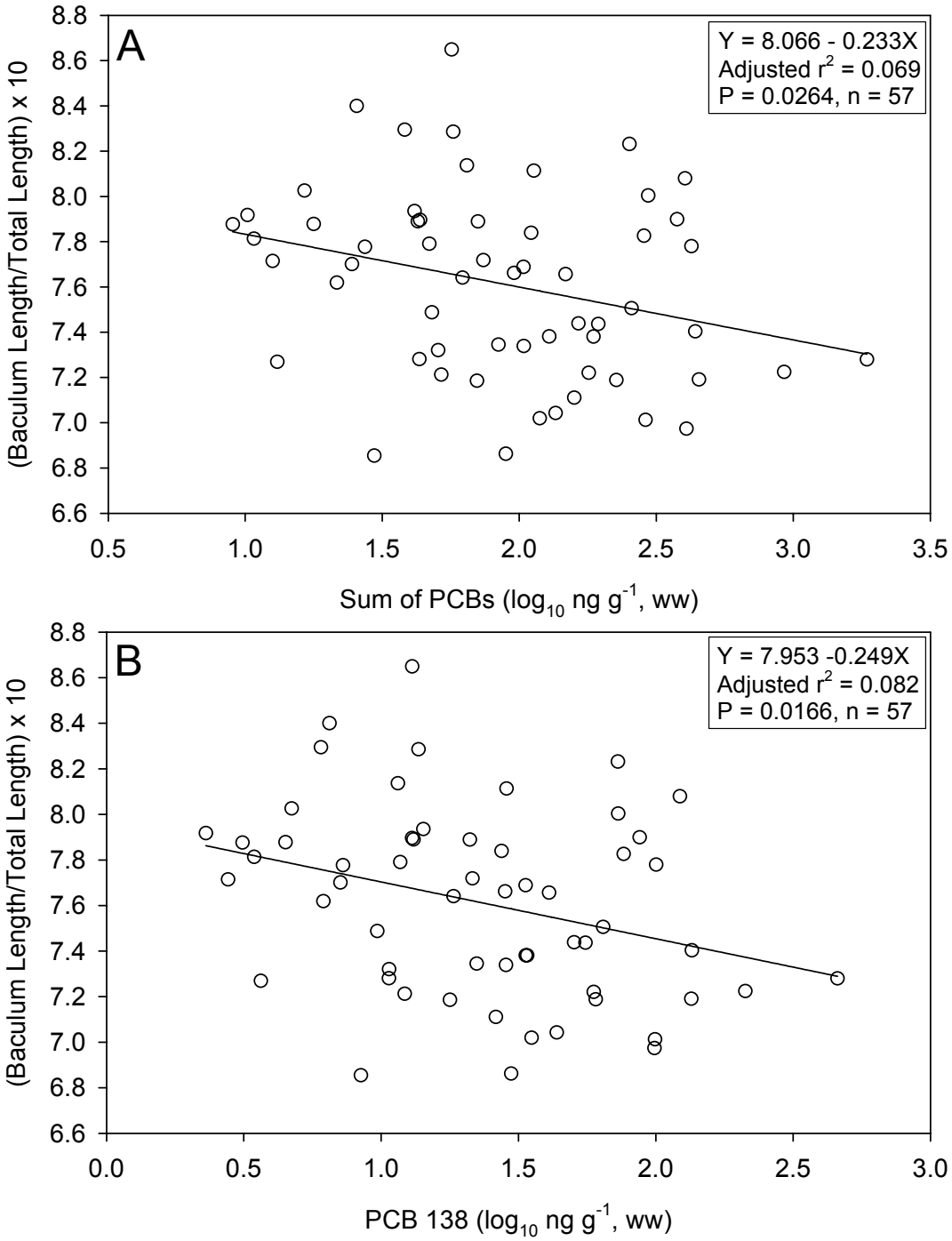


Figure 41. Relationships between (baculum length (cm)/total body length (cm)) x 10 and sum of PCBs (A) and PCB 138 (B) analyzed in livers of juvenile river otter males collected from Oregon and Washington, 1994-99.

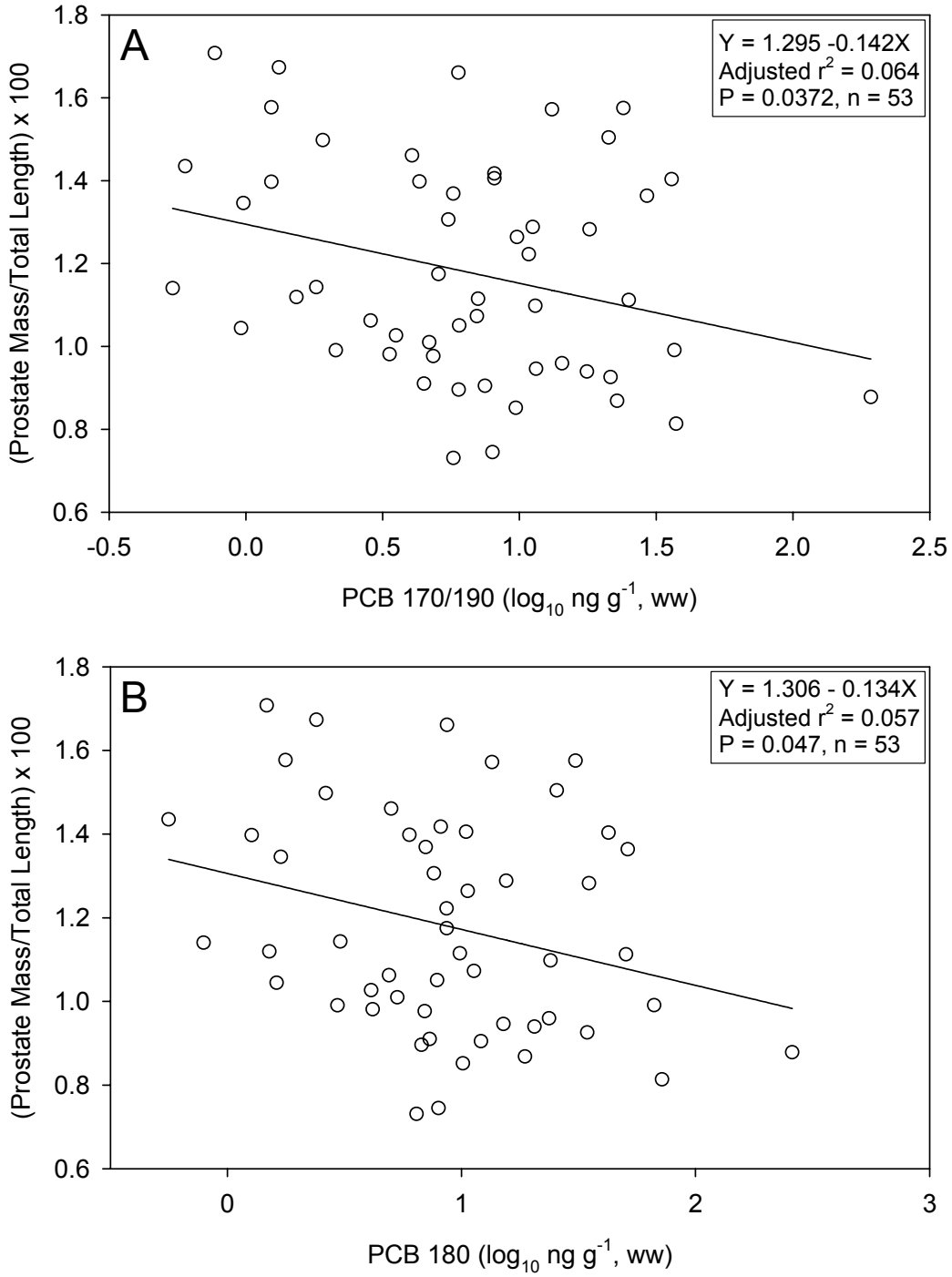


Figure 42. Relationships between (prostate mass (g)/totalbody length (cm)) x 100 and PCB 170/190 (A) and PCB 180 (B) analyzed in livers of juvenile river otter males collected from Oregon and Washington, 1994-99.

Table 28. Multiple regression equations showing the relationship between $\log_{10}$ OC insecticides and metabolites and PCBs with baculum length and mass of adult male river otters collected from Oregon and Washington, 1994-99. Ages included four year olds and older as the baculum is still growing at age three.

	Multiple Regression				
Contaminant	r^2	Slope	P	P Total Length	P Contaminant
Baculum Length (mm) n = 45					
PCB 52	0.3742	-	<0.0001	<0.0001	0.0140
PCB 99	0.3579	-	<0.0001	<0.0001	0.0257
PCB 101	0.3448	-	<0.0001	<0.0001	0.0419
PCB 129	0.3714	-	<0.0001	<0.0001	0.0155
PCB 146	0.3494	-	<0.0001	<0.0001	0.0353
PCB 153	0.3199	-	0.0001	<0.0001	0.1076
PCB 172	0.3339	-	<0.0001	<0.0001	0.0631
PCB 200	0.3219	-	0.0001	<0.0001	0.0995
Baculum Mass (g) n = 45					
HCB	0.1484	-	0.0129	0.0310	0.0309
DDE	0.2212	-	0.0020	0.0041	0.0038
DDD	0.2377	-	0.0013	0.0235	0.0023
Oxychlordane	0.1253	-	0.0226	0.0164	0.0596
trans-Nonachlor	0.2085	-	0.0028	0.0126	0.0055
Heptachlor Epoxide	0.1527	-	0.0118	0.0120	0.0279
Dieldrin	0.1803	-	0.0058	0.0127	0.0125
PCB 52	0.2384	-	0.0012	0.0125	0.0023
PCB 146	0.1094	-	0.0330	0.0124	0.0942
PCB 171	0.1076	-	0.0345	0.0116	0.0995

P = the p value of the multiple regression test with alpha = 0.10.

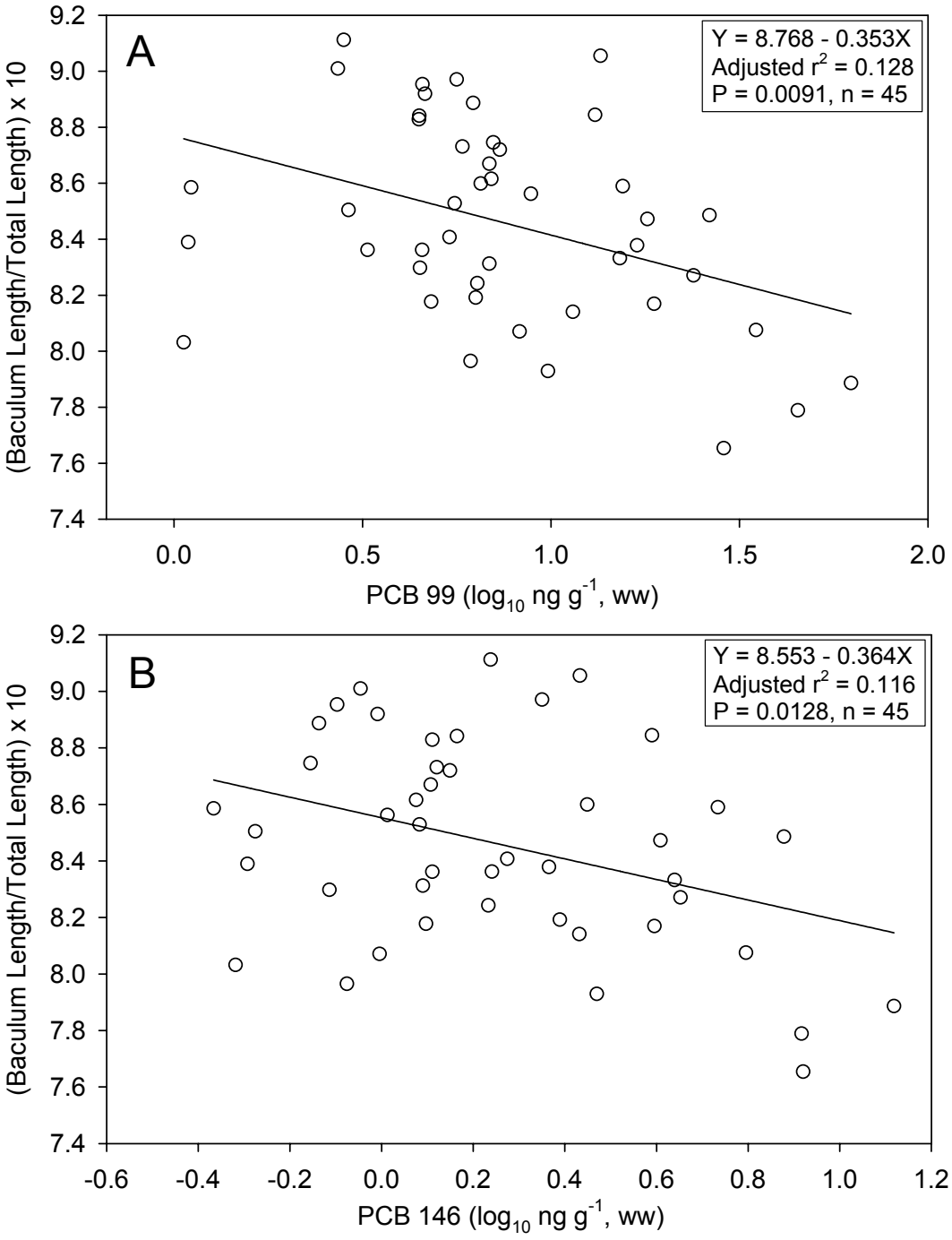


Figure 43. Relationships between (baculum length (mm)/total body length (cm)) x 10 and PCB 99 (A) and PCB 146 (B) in livers of adult river otter males collected from Oregon and Washington, 1994-99. Ages included four year olds and older as baculums are still growing until the age of three.

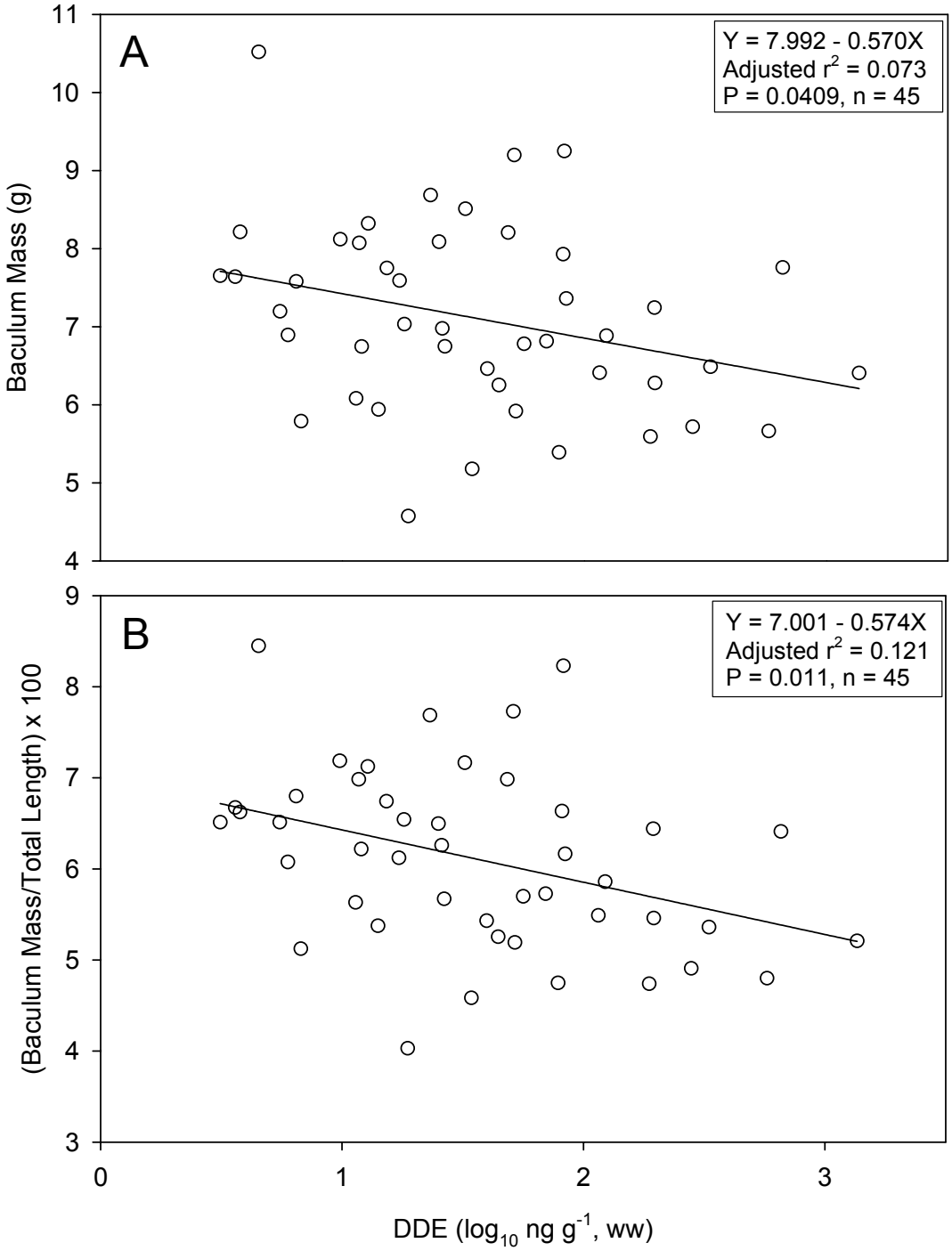


Figure 44. Relationships between baculum mass in g (A) and (baculun mass (g)/ total body length (cm)) x 100 (B) and DDE concentrations in livers of adult river otter males collected from Oregon and Washington, 1994-99. Ages included four year olds and older as baculums are still growing until the age of three.

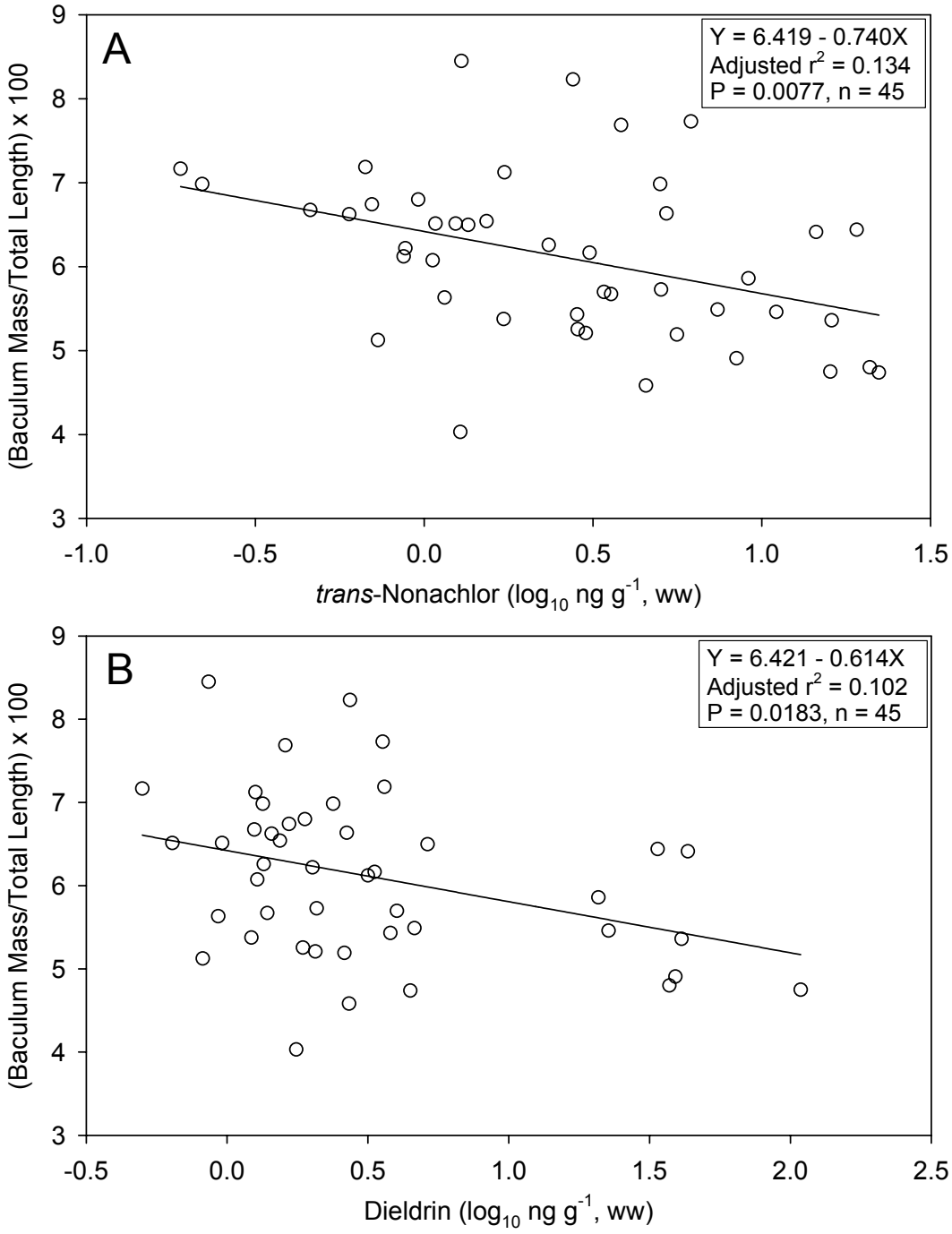


Figure 45. Relationships between (baculum mass (g)/total body length (cm)) x100 and *trans*-nonachlor (A) and dieldrin (B) in livers of adult river otter males collected from Oregon and Washington, 1994-99. Ages included four year olds and older as baculums are still growing until the age of three.

DISCUSSION

Though animal studies under controlled laboratory conditions are essential in determining dose-response relationships and molecular mechanisms of toxicity, they reveal nothing about the dynamic biological responses resulting from chronic exposure to environmental concentrations of contaminant mixtures (Beeby 2001, Fox 2001). Not only do wildlife field studies provide information on the categories, concentrations and bioavailability of environmental contaminants, they also yield important information on the interactive effects of contaminants with other environmental factors that define the final toxicological response to a wildlife species. Data resulting from field studies like this provide the basis for hypotheses generation (Fox 2001). As with all environmental contaminant studies dealing with wildlife, one needs to understand the biology and general health status of an animal species/population used in a particular study in order to evaluate possible deleterious effects associated with contaminant body burdens. In contrast to humans, few data exist for most free-living wildlife species on reproductive success, general health, disease and deformity prevalence, sex ratios and biological morphometrics. In fact, it is only after a population almost disappears that notice is given to its status and cause of decline. Therefore, we underestimate the occurrence, prevalence and severity of contaminant effects on impacted wildlife populations because the animals we study are what remains.

Mink and river otter are considered important wildlife resources, both economically and aesthetically (Calabrese et al. 1992, Halbrook et al. 1996). Their position at the top of the food chain and sensitivity to certain environmental contaminants make them good candidates as indicator or sentinel species for monitoring aquatic environments. This study began as a field evaluation of mink and otter populations on the LCR relative to persistent environmental contaminants in 1994-95. The otter population was found to be relatively dense and well distributed throughout the LCR (Henny et al. 1996). Otters were found and trapped regularly throughout the study area, whereas mink were disturbingly

absent (Henny et al. 1996). The mink population, whose harvest declined severely in numbers over the last 40 or more years (Henny et al. 1981), was nearly extirpated though the habitat for many portions of the Columbia River was found to be excellent (Henny et al. 1996). It is well known that mink are extremely sensitive to PCBs and other contaminants (Platonow and Karstad 1973, Aulerich and Ringer 1977, Jensen et al. 1977, Bleavins et al. 1980, Hochstein et al. 1988, Leonards et al. 1994, Heaton et al. 1995) and may be responsible for their absence in contaminated portions of the Columbia River Basin. Based on river otter and mink residue data collected in 1978-79 from the LCR (Henny et al. 1981), PCB concentrations are much lower than they were in the late 1970s. However, PCB concentrations in mink from the LCR during the late 1970s were equivalent to mink that survived long-termed PCB dosage studies, but failed to produce kits that survived (Heaton et al. 1995). Henny et al. (1981) reported that the river otter collected contained much higher PCB concentrations than mink at that time and were still relatively dense in numbers, suggesting that otters are less sensitive. Therefore, the otter was chosen for continued study because of its regional availability and possible reduced sensitivity to organochlorine contaminants when compared to mink. The study area was expanded to include the states of Oregon and Washington. A total of 303 otters were collected from a variety of habitats including relatively clean areas, but also from heavily contaminated areas. This approach of using animals with varied exposure to contaminants provided an opportunity to better evaluate the juvenile male reproductive organ hypoplasia observed earlier, and more fully evaluate if the phenomenon was contaminant related, and if so, what contaminants.

Contaminants

Metals

Arsenic is an important environmental toxicant which can exist in both inorganic and organic forms, either in a trivalent (arsenite) or pentavalent

(arsenate) oxidation state (Akter et al. 2005). Arsenites are 60 times more toxic than arsenates, with organic forms of As less toxic than inorganic forms. Arsenic is found in various types of geologic rocks and soils. But, most sources of As contamination are a result of mining, smelting, some pesticides and wood preservation treatment. Soil As concentrations in Oregon and Washington States range from 1 to 10 μg g^{-1} (dw) (Boerngen and Shackette 1981, Juan 1994, Baldwin and McCreary 1998), with freshwater sediment As concentrations between 3 to 16 μg g^{-1} (dw)(Harrison et al. 1995). Normal background arsenic concentrations are < 1 μg g^{-1} (ww) in living tissues associated with terrestrial and freshwater biota (Eisler 2000f). Higher tissue concentrations are usually associated with natural sources, mine tailings, smelters, geothermal activity, arsenical pesticides and marine environs. Marine organisms normally exhibit As concentrations from several to 100 μg g^{-1} (dw) (Lunde 1977). Marine biota As concentrations though, are usually in the form of organic arsenicals, unless near anthropogenic sources. Arsenic concentrations found in otter livers from this study did not exceed 8.5 μg g^{-1} (dw), but the range of concentrations found were higher than those reported for several species of marine mammals (Kubota et al. 2001, Kubota et al. 2003). No As data or cases of As poisoning have been reported in the literature for the mink, Eurasian otter or river otter. All but four of the adult river otter with quantifiable liver concentrations of As were associated with coastal or marine environs of Oregon and Washington.

Cadmium, as cadmium oxide, enters the environment naturally through rock weathering and volcanic activity. But, most Cd enters the environment through human activities of mining and smelting (especially zinc), fuel combustion, electronics production and disposal of metal containing products (Eisler 2000a). Cadmium is not eliminated well from the body and tends to accumulate with age. Cadmium concentrations have been reported from several parts of North America for river otters (Anderson-Bledsoe and Scanlon 1983, Wren et al. 1988, Halbrook et al. 1996, Harding et al. 1998), ranging between nd to 1.58 μg g^{-1} (dw). Cadmium concentrations in adult river otter livers from this study fell within the reported range, with the highest concentration at 1.18 μg g^{-1}.

Adult river Cd mean and individual concentrations were lower than the majority of means and ranges reported for the Eurasian otter (Appendix 4). There was no sign of Cd accumulation with age except between juvenile and adult, and the concentrations were not sufficient to produce toxic effects (Wren et al. 1988, Harding et al. 1998).

Copper and Zn are both essential elements for normal growth and metabolism, but can be toxic at higher concentrations. Liver concentrations of Cu and Zn in juvenile and adult otters were comparable to the range of concentrations reported for otters in the literature (Anderson-Bledsoe and Scanlon 1983, Wren et al. 1988, Halbrook et al. 1996, Harding et al. 1998) and well below toxicity concentrations reported for other mammalian species in the literature (Eisler 2000b, Eisler 2000e).

Only one case of Hg poisoning has been reported in wild river otters, which happened in 1979 downstream of a pulp and paper mill in Northern Ontario, Canada where the mill was using mercury as a slimicide (Wren 1985). Total Hg in the otter liver was 96.0 μg g^{-1}, ww (~331 μg g^{-1}, dw). Other otters and mink were found dead in the same vicinity by another trapper, but tissues were not analyzed for Hg. It is probable that mortality due to Hg poisoning was prevalent among mink and otter populations during the period of Hg discharge at the mill (Mason and Wren 2001). An experimental toxicity study dosing otters with methylmercury (MeHg) at 2, 4, 8 μg g^{-1} (ww) showed a mean survival time of 184, 117 and 54 days, respectively (O'Connor and Nielson 1981). The mean liver concentration at death for total Hg was 33 μg g^{-1}, ww (~113 μg g^{-1}, dw), despite dietary differences in Hg. This study agreed with a mink Hg toxicity study (Wobeser et al. 1979), and suggested a build-up of MeHg to a critical concentration before overt toxicity was evident.

Organomercury compounds are more toxic than inorganic forms, especially MeHg (Eisler 2000c). The primary toxic effect of Hg is on the central nervous system, though other organ systems (i.e., kidney) are affected as well. Histologic findings of river otters dosed with MeHg consisted of neuronal

Table 29. Regional river otter total liver mercury concentrations (μg g^{-1}) reported on a wet and dry weight basis. Conversion between wet and dry mass was done using 71% moisture content in liver samples from this study. Values in bold are actual values reported. Age and sex were combined unless noted otherwise. This study used only male otters.

Location	n	Dry Mass (SE)	Wet Mass (SE)	Source
Clay lake - Wabigoon River, Ontario, Canada	1	331	**96.0**	Wren 1985
Ware County, Georgia, USA	6	31.3	**9.16 (2.07)**	Halbrook et al. 1994
Echols County, Georgia, USA	4	17.6	**5.11 (1.30)**	Halbrook et al. 1994
Winnipeg River, Manitoba, Canada	8	15.4	**4.46 (0.84)**	Kucera 1983
James Bay Territory, Quebec, Canada	153	13.97	**4.05 (0.28)**	Fortin et al. 2001
Western Washington, USA[b]	5	**13.8 (1.20)**	**2.08 (1.05)**	**This Study**
Olympic Peninsula, Washington, USA[b]	18	**12.6 (1.21)**	**2.11 (1.06)**	**This Study**
English River, Ontario, Canada	20	12.0	**3.47 (0.92)**	Wren et al. 1986
Whiteshell, Manitoba, Canada[d]	2	11.7	**3.38 (1.70)**	Kucera 1983
Wisconsin, USA	49	11.5	**3.34 (0.59)**	Sheffy and Amant 1982
Tadenac Lake Watershed, Ontario, Canada	4	10.3	**3.0 (1.1)**	Wren 1984
Southwest Washington, USA[b]	16	**10.0 (1.21)**	**1.97 (1.06)**	**This Study**
Willamette River Basin, Oregon, USA[b]	17	**9.26 (1.17)**	**1.88 (1.05)**	**This Study**
Oregon Coast, USA[b]	17	**9.23 (1.15)**	**1.92 (1.04)**	**This Study**
Keweenaw Peninsula, Michigan, USA[b]	6	8.69	**2.52 (0.21)**	Francis and Bennett 1994
Olympic Peninsula, Washington, USA[c]	7	**8.05 (1.23)**	**1.83 (1.06)**	**This Study**
Puget Sound, Washington, USA[b]	12	**7.89 (1.27)**	**1.81 (1.07)**	**This Study**
West Adirondack Mountains, New York, USA	14	7.86	**2.28**	Foley et al. 1988
Southwest Washington, USA[c]	14	**7.84 (1.13)**	1.80	**This Study**
New York, USA[b]	62	7.24	**2.10 (0.15)**	Yates et al. 2005
Turkey Lake, Ontario, Canada	8	6.86	**1.99 (0.18)**	Wren et al. 1986
Southeastern Ontario, Canada	34	**6.7 (0.70)**	1.94	Mierle et al. 2000
Eastern Lake Plains, New York, USA	6	6.55	**1.90**	Foley et al. 1988

Table 29. Continued.

Location	n	Dry Mass (SE)	Wet Mass (SE)	Source
Northeast Adirondack Mountains, New York, USA	12	6.34	**1.84**	Foley et al. 1988
Maine, USA[b]	41	6.17	**1.79 (0.16)**	Yates et al. 2005
Wekusko, Manitoba, Canada[d]	10	6.13	**1.78 (0.28)**	Kucera 1983
Massachusetts, USA	103	6.07	**1.76**	Organ 1989
Western Washington, USA[c]	6	**5.85 (1.24)**	**1.67 (1.06)**	**This Study**
New York, USA[c]	84	5.72	**1.66 (0.10)**	Yates et al. 2005
Willamette River Basin, Oregon, USA[c]	8	**5.55 (1.28)**	**1.61 (1.07)**	**This Study**
South-Central Ontario, Canada	41	5.5	**1.61 (0.12)**	Evans et al. 2000
Herschel Township, Ontario, Canada	19	5.31	**1.54 (0.17)**	Evans et al. 1998
Oregon Coast[c]	8	**5.26 (1.18)**	**1.60 (1.06)**	**This Study**
Muskoka, Ontario, Canada	12	5.17	**1.50 (0.22)**	Wren et al. 1986
Keweenaw Peninsula, Michigan, USA[c]	5	4.86	**1.41 (0.20)**	Francis and Bennett 1994
Lower Columbia River, Oregon, USA[a]	11	4.74	**1.37 (0.41)**	Henny et al. 1996
Hudson River, New York, USA	2	4.52	**1.31**	Foley et al. 1988
Puget Sound, Washington, USA[c]	12	**3.74 (1.21)**	**1.45 (1.05)**	**This Study**
Lower Columbia River, Oregon, USA[c]	6	**3.63 (1.19)**	**1.46 (1.05)**	**This Study**
Lower Columbia River, Oregon, USA[b]	9	**3.46 (1.14)**	**1.45 (1.04)**	**This Study**
Lower Fraser River, British Columbia, Canada	3	**3.50 (1.72)**	1.02	Harding et al. 1998
Upper Columbia River, British Columbia, Canada	4	**3.31 (1.19)**	0.96	Harding et al. 1998
Sudbury, Ontario, Canada	36	2.97	**0.86 (0.15)**	Wren et al. 1986
Maine, USA[b]	19	2.93	**0.85 (0.10)**	Yates et al. 2005
Upper Fraser River, British Columbia, Canada	6	**2.93 (0.64)**	0.85	Harding et al. 1998
Upper and Northern Lower Peninsulas, Michigan, USA	43	**2.17 (0.25)**	0.63	Ropek and Neely 1993
Kootenay River, British Columbia, Canada	12	**2.07 (0.53)**	0.60	Harding et al. 1998

[a] Yearling otters, [b] Adult otters, [c] Juvenile otters, [d] Male otters only

necrosis, astrogliosis, perivascular cuffing, demyelination, vacuolation of neuropil and malacia (O'Connor and Nielson 1981). Brain lesions were diffuse and bilateral, primarily restricted to cerebellum and cerebral cortex with some involvement of the midbrain and brainstem. Recent research has shown that $HgCl_2$ and MeHg inhibits natural ligand binding of muscarinic acetylcholine and dopaminergic receptors in river otter brains (Basu et al. 2005a, Basu et al. 2005b). The second study (Basu 2005b) demonstrated that $HgCl_2$ was a more potent inhibitor of muscarinic acetylcholine receptors than MeHg, and that the receptors of the cerebellum are more sensitive to Hg-mediated muscarinic acetylcholine receptor inhibition than with the same receptors of the cerebral cortex (Basu 2005b). By rank, the river otter was also found to be the most sensitive to Hg-mediated inhibition, followed by the rat, mink, mouse and human, regardless of Hg type and brain region (Basu 2005b).

River otters from Oregon and Washington have some of the higher liver Hg concentrations reported for North America (Table 29), but at the lower end of mean Hg concentrations reported for the Eurasian otter (Appendix 4). The Hg liver concentration of 148 μg g^{-1} from an apparently healthy adult male otter from the Olympic Peninsula is one highest reported for either the river otter or the Eurasian otter. The higher mean Hg concentrations in this study are not unexpected as cinnabar (HgS) or mercury ore deposits are interspersed throughout the western portions of Oregon and Washington. In fact, several cinnabar mines operated in both states producing metallic mercury on site using inefficient retort furnaces, thus creating hundreds of thousands of cubic yards of Hg contaminated mine tailings (Curtis 2003). Natural deposits, Hg Mining and smelting, Hg amalgamation in gold and silver mining, Hg from coal fired power generation, Hg use as a slimicide in pulp and paper mills and atmospheric transport and deposition of Hg to remote areas are important Hg sources for both Oregon and Washington (Park and Curtis 1997, Hygelund et al. 2001, Curtis 2003, Hylander and Meili 2003, Pacyna et al. 2003). As the river otter is primarily piscivorous, fish provide an important source of Hg exposure. For example, fish

collected along the Willamette River in 1993 for an osprey study (Henny et al. 2003) provides fish contaminant data for a major portion of the river, excluding the lower portion below Newberg. Mean Hg concentrations reported for fish from the Willamette River were 0.31, 1.17 and 0.11 μg g^{-1} (dw) for the largescale sucker, northern pikeminnow, and mountain whitefish, respectively (Henny et al. 2003). When considering the relative biomass of the three key fish species consumed by the osprey, the same species can contribute substantially to the otter's diet as well and therefore contaminant accumulation. Otters from Oregon and Washington had fairly high Hg liver concentrations, with one otter liver concentration (148 μg g^{-1}) exceeding the 113 μg g^{-1} found lethal to otters in a MeHg dosing study. Mercury is taken up in otters and other predatory mammals (i.e., marine mammals, mink) primarily as MeHg, and is transformed into a less toxic inorganic form of mercury. Only total Hg was analyzed in this study, thus it is not known what the percentage of MeHg was present in the liver samples analyzed. The percentage of MeHg to total Hg; however, was probably low due to demethylation as reported in other wildlife species. It is not known if neurological damage was occurring in this species at the concentrations reported; no nerve tissue samples were taken for histological evaluation. Metallothioneins (i.e., selenomethionine), selenite, glutathione and other factors may be protective against Hg toxicity, but mild to moderate nerve tissue damage cannot be ruled out (Wang et al. 2001, Frisk et al. 2003, Decataldo et al. 2004, Henkal and Krebs 2004, Huang et al. 2004, Ikemoto et al. 2004).

Tributyltin has been used for over 30 years as an antifouling agent in marine paint formulations to prevent the accumulation of barnacles and slime on boat and ship hulls (Kannan et al. 1999). Concern developed over the use of TBT and its degradation products DBT and MBT in the 1980s because TBT had deleterious effects (i.e., imposex) on several marine organisms (mostly bivalves and gastropods) at very low concentrations (Bryan et al. 1986, Alzieu et al. 1990, Horiguchi et al. 1994), . Based on many laboratory studies reporting sub-lethal and lethal effects of TBT, many countries (including the USA) passed legislation

limiting the use of TBT-based paints on boats and ships in the late 1980s (Uhler et al. 1993, Fent 1996). Recent studies report that butyltins can accumulate at higher trophic levels of the food chain in tissues of marine birds and mammals (Iwata et al. 1995, Guruge et al. 1996, Kannan et al. 1996, Kim et al. 1996a, Kim et al. b, Kannan et al. 1997, Tanabe et al. 1998). Tributyltin is toxic to mammals with an LD_{50} in rats of 122 - 194 μg g^{-1} (USEPA 1997) and is a strong inhibitor of mitochondrial oxidative phosphorylation (Eisler 2000d). Several types of neuronal toxicity are also caused by TBT exposure (Feldman et al. 1993, Nakatsu et al. 2006). Tributyltin is also known to induce chromosomal aberrations in mammals (Jensen et al. 1991, Sasaki et al. 1993) and inhibits CYP1A and aromatase activity (Heidrich et al. 2001, Morcillo et al. 2004).

Significantly higher concentrations of butyltins were found in otter livers from the PS, while the lowest concentrations were found associated with small freshwater streams and lakes. The higher tissue concentrations of butyltins in otters living in the PS suggests elevated exposure due to the shipping activity present with greater availability of TBT in seawater compared to freshwater (Kannan et al. 1999). Butyltins, particularly TBT exist as hydroxides and chlorides in waters at or above the pH of eight, whereas butyltins in waters of lower pH exist in the cation form (Fent and Looser 1995, Fent et al. 1995, Stäb et al. 1996). Neutral butyltin forms are more bioavailable and readily accumulated in aquatic biota than are the cation forms. Few studies have reported butyltin contamination of water, sediment, and biota from Oregon and Washington. Total butyltin concentrations found in bivalve mollusks from Grays Harbor and Willapa Bays of Washington in 1989-90 were 100 and 4 ng g^{-1}(ww), respectively, while mollusks collected from the LCR and Yaquina Bay, Oregon, had 94 and 56 ng g^{-1} (ww), respectively (Uhler et al. 1993). Butyltin concentrations in fish collected at Elliott Bay, Washington, were 290-380 ng g^{-1} (ww), whereas fish from Coos Bay, Oregon were 690 ng g^{-1} (ww) (Krone 1996). While butyltin contamination has been documented in coastal regions, studies of butyltin contamination in freshwater systems are scarce. Otter liver concentrations of butyltins in

freshwater systems from Oregon and Washington suggest contamination of these systems as well.

Among the butyltins compounds analyzed, DBT and MBT were the major forms found in river otter livers. Hepatic DBT contributed the greater proportion of total butyltin concentrations of several species of marine mammals which can explained by the metabolic transformation of TBT to DBT and MBT in marine organisms (Lee 1991). TBT was a small percentage (1.5 - 5.9%) of the total butyltin in river otter livers from all areas sampled (Table 19), which was less than the 7 to 45 TBT percentage found in marine mammals (Iwata et al. 1995, Kim et al. 1996a, Kim et al. 1996b, Kannan et al. 1997, Tanabe et al. 1998). Efficient metabolic transformation of TBT to DBT and MBT, and exposure to BTs with a greater percentage of DBT and MBT could explain the abundance of DBT and MBT in river otter livers. However, river otter exposures to DBT and MBT in freshwater settings could be originating from leaching due to other sources as the two compounds are also used as stabilizers in the production of PVC pipe (Maguire et a. 1982, Fent and Müller 1991, Chau et al. 1992).

Hepatic butyltin concentrations in river otters from the PS were comparable to concentrations reported for cetaceans and marine mammals along the coastlines of Japan and the United States, but otters from freshwater systems were much lower (Iwata et al. 1995, Kim et al. 1996a, Kim et al. 1996b, Kannan et al. 1997, Tanabe et al. 1998). As most river otters are found in freshwater environs, they are not expected to have BT concentrations at the levels reported for marine mammals. Elimination of BTs through molting of fur and hair has been reported in the Stellar's sea lion (Kim et al. 1996a).

OC Insecticides and Metabolites

Mean hepatic organochlorine insecticide and metabolite concentrations for both juvenile and adult river otter males were all below 100 μg kg^{-1}. The higher mean OC concentrations were associated with the LCR and WB, indicative of regional (agriculture) contaminant sources. Only DDE, DDD and HCB had

individual concentrations exceeding 500 μg kg^{-1}. Mean concentrations of DDE and DDD have declined dramatically from those reported by Henny et al. (1981) for LCR and OC otters collected in 1978-79. Mean concentrations DDE and DDD of otters collected away from agricultural and industrial contaminant sources (OC, OP, SW, WW) were comparable with otters collected from the upper Columbia and Fraser River Basins of British Columbia (Elliott et al. 1999). Mean DDE and DDD concentrations from the LCR were also lower than composite otter liver samples (680, 4700, 150 μg kg^{-1} for DDE; 47.0, 30.0, 7.70 μg kg^{-1} for DDD; n = 3, 3, 10, respectively) collected from the same region in 1990-91 (Elliott et al. 1999). However, river otter liver means associated with regional and point contaminant sources in this study (i.e., agriculture in the Willamette River Basin and the Wenatchee, WA area, and agricultural and industrial sources in the Portland, OR region) are still some of the higher DDE values reported in the literature for this species (Table 30). The higher regional DDE means from this study are at the lower end of means reported for the Eurasian otter (Appendix 5).

Mean hepatic dieldrin concentrations for both juvenile and adult male river otters were below 5 μg kg^{-1}, while dieldrin concentrations ranged between 0.12 to 52.3 μg kg^{-1} for juveniles and 0.25 to 267 μg kg^{-1} for adults. A paucity of data pertaining to dieldrin concentrations in river otters exists in the literature, so few comparisons could be made. However, dieldrin means for juvenile and adult male otters from the LCR are lower than composite liver concentrations (17.0, 71.0, 6.1 μg kg^{-1}, n = 3, 3, 10, respectively) reported for otters collected from the same region in 1990-91 (Elliott et al. 1999), showing a significant decline in dieldrin concentrations within a time frame of less than five years. Dieldrin concentrations reported in livers from this study for both juvenile and adult river otters were significantly higher than reported in northeastern Alberta (Somers et al. 1987) and the upper Columbia and Fraser Rivers of British Columbia, Canada (Harding et al. 1999, Elliott et al. 1999). Mean dieldrin concentrations from this

study were also at the lower end of concentrations reported for the Eurasian otter (Appendix 6).

Dieldrin is considered by some researchers in Europe to be the main contaminant responsible for the decline of Eurasian otter populations as well as other wildlife species (Jefferies and Hanson 2000). The use of dieldrin as a seed dressing in England began around 1956. Later, dieldrin was used as a sheep dip. Many wildlife species, including mammals, died from dieldrin intoxication (DeWitt et al. 1960, Turtle et al. 1963, Jefferies et al. 1973, Newton and Haas 1988). Foxes were found to be extremely sensitive to dieldrin, with tissue concentrations above 1 μg g^{-1} (ww) considered lethal. Some 1,300 red fox died in the winter of 1959-60 in the eastern counties of England, with dieldrin found to be the cause of death (Taylor and Blackmore 1961, Blackmore 1963). Eurasian otter declines were correlated with the initiation of dieldrin use and subsequent wildlife die-offs, with the majority of otter mortality associated with the more arable lands of south and east England (Jefferies and Hanson 2000). Dieldrin use in the United States and Canada was not as pronounced as in England and other parts of Europe. River otter liver concentrations of dieldrin from this study were certainly well below concentrations suggested to cause problems with Eurasian otters.

PCBs

Mean hepatic ΣPCB concentrations for both juvenile and adult male otters were all under 1 μg g^{-1} for all regional locations, with the highest ΣPCB means associated with the more populated areas of Oregon and Washington (i.e., LCR, PS, WB, WW). Adult mean PCB concentrations both regionally and as a whole were generally double that of the juvenile means. The thirteen individual otters with hepatic ΣPCB concentrations greater than 1 μg g^{-1} were all located near large metropolitan areas or known point sources of PCB contamination, except for the 2 adult male otters collected outside of Woodinville, Washington along Bear Creek. No published sources of PCB contamination were found for the

vicinity where the two otters were trapped; however, industrial activity was noted adjacent to the creek.

Most published data on river otter PCB tissue concentrations could not be compared directly with data from this study because the earlier PCB information was based on Aroclor standards (Table 30). PCBs are a class of synthetic halogenated aromatic hydrocarbon compounds, with a possible 209 congeners formed (Safe 1994). Commercial PCB production is a heated chlorination process of biphenyl using anhydrous chlorine in the presence of iron filings or ferric chloride as a catalyst (Eisler 2005g). The conditions by which chlorination occurs determines the percent of chlorine by weight which historically ranged between 18 and 79%. Chlorine substitution varies from one to 10 atoms at the ortho-, meta- and para- positions of the biphenyl rings. The Log K_{ow} for the suite of PCB congeners ranges of 4.6 to 9.6. Aroclors are complex mixtures of numerous PCB congeners which vary in percent chlorine by weight (Frame et al. 1996, Kodavanti et al. 2001, Blankenship et al. 2005). For example, Aroclor 1254 is 54 percent chlorine by weight as indicated by the last two digits in the numeric designation, while Aroclor 1260 is 60 percent chlorine. Figure 46 shows the difference in PCB congener composition between Aroclors 1248, 1254 and 1260. Also, Aroclor PCB congener composition is different from lot to lot because of production variations of temperature, percent chlorine present, type and amount of catalyst used and duration of the chlorination process. Figure 47 shows the difference in PCB congener profiles for four different Aroclor 1254 lots. Burgin et al. (2001) and Kodavanti et al. (2001) have shown differential responses from two Aroclor 1254 lots on enzymatic induction, thyroid hormone concentrations and oxidative stress. Tissue analyses using Aroclors as standards also do not take into account environmental changes in Aroclor congener composition due to PCB weathering via dechlorination, volatilization, environmental partitioning and preferential metabolism of specific congeners, which is a function of time (Blankenship et al. 2005). Figure 48 shows the difference in PCB congener composition between Aroclors 1248, 1254 and 1260

Table 30. Geometric mean (except where noted) DDE and PCB concentrations in river otter tissues from North America. Reported concentrations are μg/g^{-1} wet weight. PCB concentrations are based on aroclor standards except where noted.

Location	Year	Tissue	n	Mean	Range	Source
DDE						
Lower Columbia River, Oregon	78-79	Liver	7	1.65	0.43-4.80	Henny et al. 1981
Columbia River, USA	90-92	Liver	4	0.73	0.15-4.70	Elliott et al. 1999
Coastal Rivers, Oregon	78-79	Liver	3	0.21	1 nd-0.60	Henny et al. 1981
Lower Columbia River, Oregon	94-99	Liver	32	0.12	0.007-1.82	This Study
Upper Columbia River, Washington	94-99	Liver	4	0.12	0.005-1.51	This Study
Kootenay River, Canada	94-96	Liver	12	0.12	nd-1.69	Harding et al.1999
Willamette River Basin, Oregon	94-99	Liver	34	0.08	0.002-1.59	This Study
Northwest Illinois	84-89	Liver	8	0.06[b]	0.02-0.10	Halbrook et al. 1996
Upper Klamath Lake, Oregon	78-79	Liver	2	--	nd-0.10	Henny et al. 1981
Lower Columbia River, Canada	94-96	Liver	1	nd		Harding et al.1999
Upper Columbia River, Canada	94-96	Liver	4	0.04	0.02-0.08	Harding et al.1999
Columbia River, Canada	90-92	Liver	3	0.03	0.01-0.98	Elliott et al. 1999
Western Washington	94-99	Liver	12	0.03	0.0005-0.28	This Study
Olympic Peninsula, Washington	94-99	Liver	25	0.02	0.006-0.08	This Study
Puget Sound, Washington	94-99	Liver	27	0.02	0.004-0.28	This Study
Southwestern Washington	94-99	Liver	32	0.02	0.002-0.11	This Study
Oregon Coast	94-99	Liver	30	0.01	0.0003-0.19	This Study
Lower Fraser River, Canada	94-96	Liver	3	0.01	0.006-0.01	Harding et al.1999
Upper Fraser River, Canada	94-96	Liver	6	nd		Harding et al.1999
Fraser River, Canada	90-92	Liver	1	nd		Elliott et al. 1999
Northeastern Alberta, Canada	80-83	Liver	44	0.002[b]	nd-0.02	Somers et al. 1987
Lower Columbia River, Oregon	78-79	Muscle	7	0.79	0.48-1.50	Henny et al. 1981
Southeastern Alabama	72-74	Muscle	32	0.18	nd-3.60	Hill and Lovett 1975
Coastal Rivers, Oregon	78-79	Muscle	3	--	2 nd-0.18	Henny et al. 1981

Table 30. Continued.

Location	Year	Tissue	n	Mean	Range	Source
DDE						
Northwest Illinois	84-89	Muscle	8	0.04[b]	0.02-0.10	Halbrook et al. 1996
Upper Klamath Lake, Oregon	78-79	Muscle	2	--	nd-0.10	Henny et al. 1981
Ware County, Georgia	76-77	Fat	58	--	8 nd-91.4	Halbrook et al. 1981
Echols County, Georgia	76-77	Fat	36	--	4 nd-137	Halbrook et al. 1981
Piedmont Region, Georgia	76-77	Fat	34	--	14 nd-87.3	Halbrook et al. 1981
Northeastern Alberta, Canada	80-83	Fat	58	0.008[b]	nd-0.16	Somers et al. 1987
Northwest Illinois	84-89	Brain	8	0.04[b]	0.02-0.06	Halbrook et al. 1996
PCBs						
Lower Columbia River, Oregon	78-79	Liver	7	6.99	1.70-23.0	Henny et al. 1981
Coastal Rivers, Oregon	78-79	Liver	3	1.36	1 nd-8.40	Henny et al. 1981
Massachusetts	86-87	Liver	103	1.03	nd-22.0	Organ 1989
Columbia River, USA[a]	90-92	Liver	4	0.76	0.52-1.50	Elliott et al. 1999
Lower Columbia River, Oregon[a]	94-99	Liver	32	0.45	0.09-2.41	This Study
Northwest Illinois	84-89	Liver	8	0.34[b]	0.04-0.86	Halbrook et al. 1996
Michigan all females	85-87	Liver	51	0.30[b]	25 nd-4.40	Stuht 1991
Puget Sound, Washington[a]	94-99	Liver	27	0.29	0.01-10.1	This Study
Willamette River Basin, Oregon[a]	94-99	Liver	34	0.23	0.01-3.57	This Study
Columbia River, Canada[a]	90-92	Liver	3	0.17	0.06-0.58	Elliott et al. 1999
Western Washington[a]	94-99	Liver	12	0.16	0.03-1.62	This Study
Kootenay River, Canada[a]	94-96	Liver	12	0.15	nd-0.61	Harding et al.1999
Lower Fraser River, Canada[a]	94-96	Liver	3	0.14	nd-0.35	Harding et al.1999
Upper Columbia River, Washington[a]	94-99	Liver	4	0.13	0.009-1.07	This Study
Upper Columbia River, Canada[a]	94-96	Liver	4	0.12	nd-0.21	Harding et al.1999
Oregon Coast	94-99	Liver	30	0.07	0.02-0.27	This Study
Olympic Peninsula, Washington[a]	94-99	Liver	25	0.07	0.01-0.18	This Study
Southwestern Washington[a]	94-99	Liver	32	0.06	0.01-0.31	This Study

Table 30. Continued.

Location	Year	Tissue	n	Mean	Range	Source
PCB						
Fraser River, Canada[a]	90-92	Liver	1	0.04		Elliott et al. 1999
Upper Fraser River, Canada[a]	94-96	Liver	6	0.03	nd-0.17	Harding et al.1999
Northeastern Alberta, Canada	80-83	Liver	44	0.02[b]	nd-0.08	Somers et al. 1987
New York	82-84	Liver	45	--	0.04-7.27	Foley et al. 1988
Upper Klamath Lake, Oregon	78-79	Liver	2	--	nd-2.00	Henny et al. 1981
Lower Columbia River, Canada[a]	94-96	Liver	1		1.44	Harding et al.1999
Lower Columbia River, Oregon	78-79	Muscle	7	3.24	1.10-8.30	Henny et al. 1981
Southeastern Alabama	72-74	Muscle	19	--	14 nd-2.50	Hill and Lovett 1975
Coastal Rivers, Oregon	78-79	Muscle	3	--	2 nd-0.50	Henny et al. 1981
Upper Klamath Lake, Oregon	78-79	Muscle	2	--	nd-1.60	Henny et al. 1981
Northwest Illinois	84-89	Muscle	8	0.16[b]	0.02-0.32	Halbrook et al. 1996
New York	82-84	Fat	45	--	0.06-114	Foley et al. 1988
Echols County, Georgia	76-77	Fat	36	--	18 nd-66.7	Halbrook et al. 1981
Michigan	81-82	Fat	39	3.18[b]	3 nd-38.5	Stuht 1991
Ware County, Georgia	76-77	Fat	58	--	29 nd-32.1	Halbrook et al. 1981
Piedmont Region, Georgia	76-77	Fat	34	--	8 nd-31.0	Halbrook et al. 1981
Northeastern Alberta, Canada	80-83	Fat	58	0.38[b]	nd-2.34	Somers et al. 1987
Northwest Illinois	84-89	Brain	8	0.27[b]	0.02-0.85	Halbrook et al. 1996

[a] PCBs based on sum of congeners analyzed.

[b] Arithmetic mean.

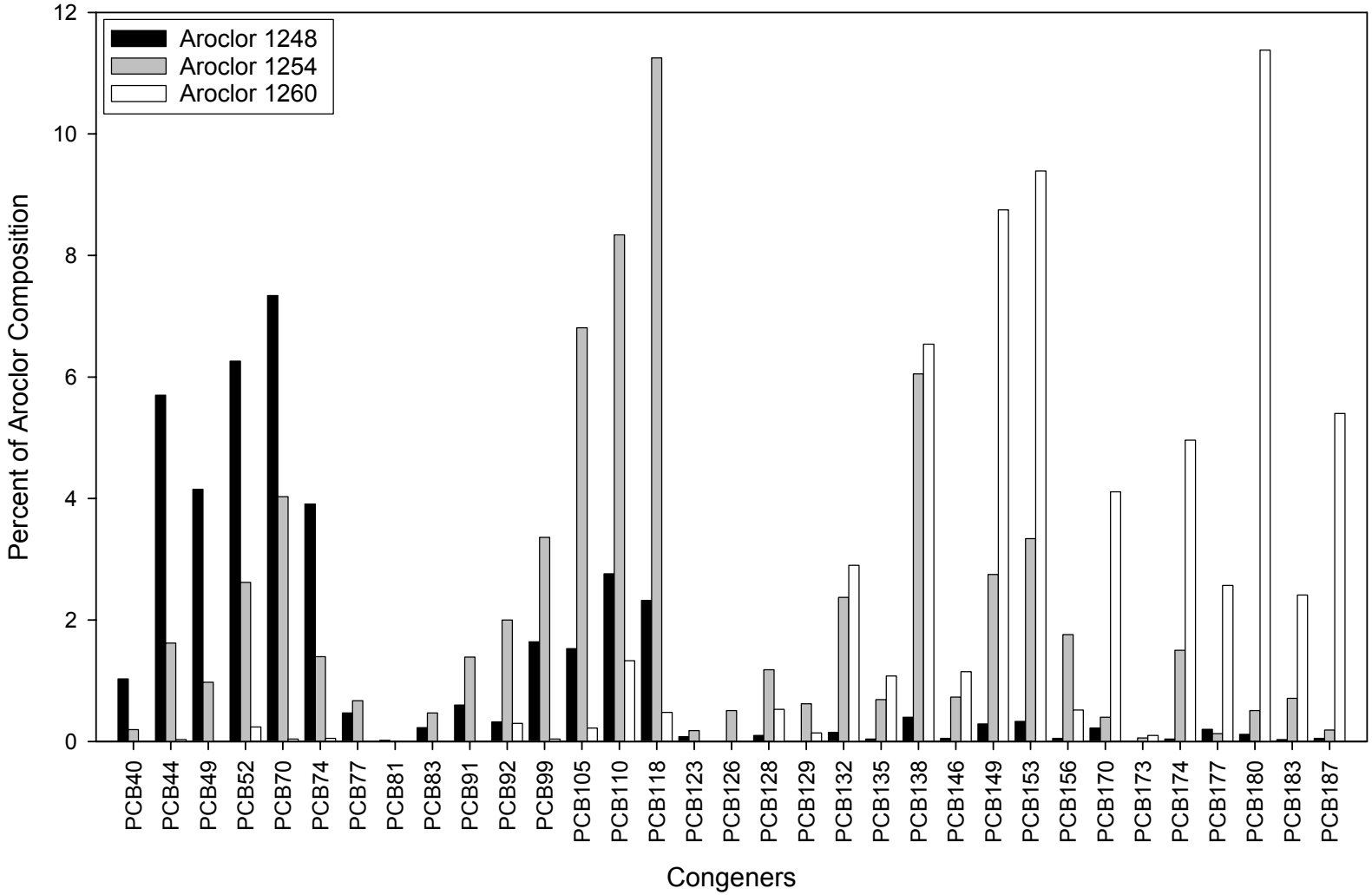


Figure 46. Comparison between congener profiles of Aroclors 1248, 1254 and 1260. Aroclor congener data taken from Frame et al. (1996).

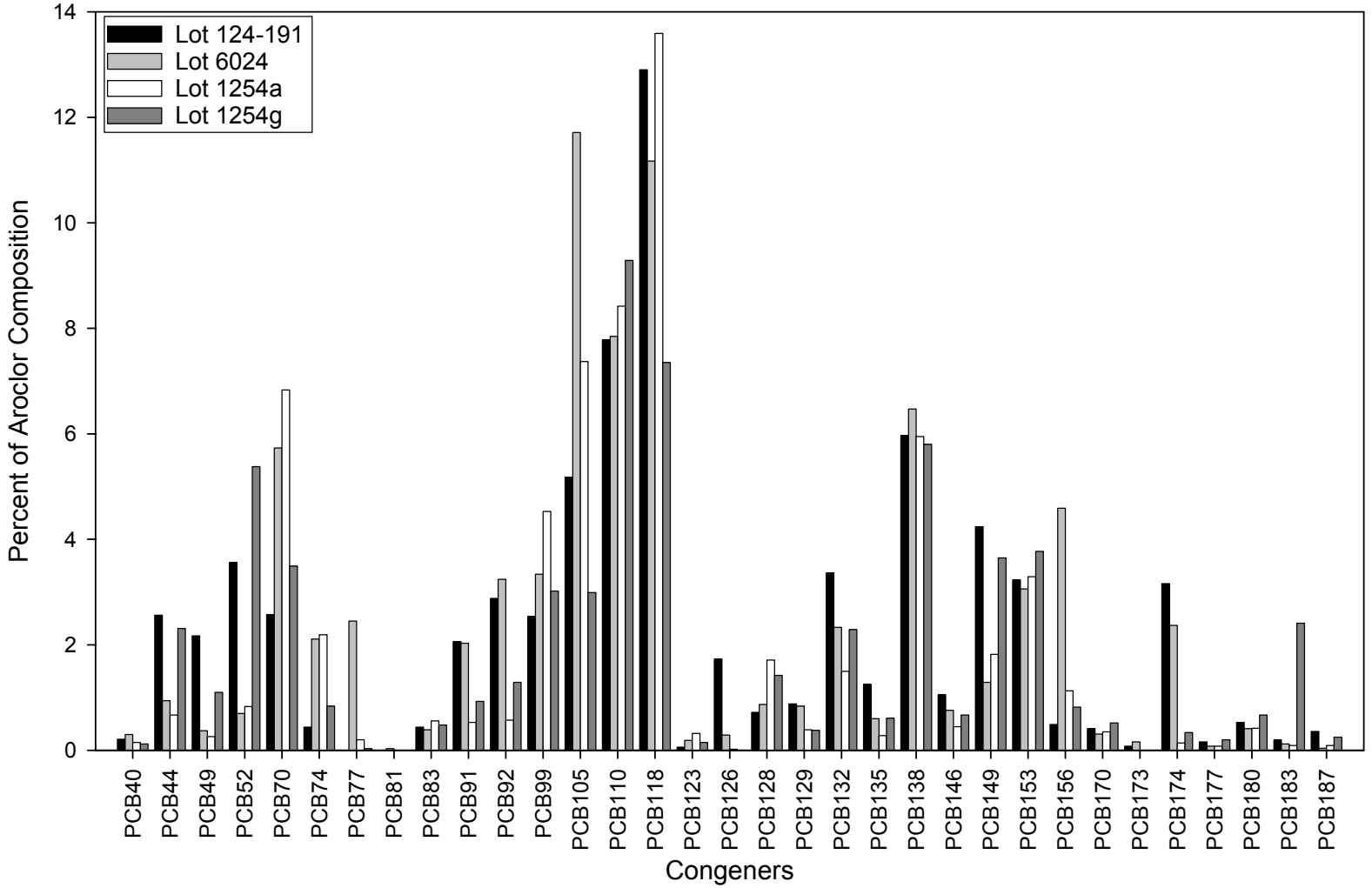


Figure 47. Comparison between congener profiles of four Aroclor 1254 lots. Aroclor congener data taken from Frame et al. (1996) and Kodavanti et al. (2001).

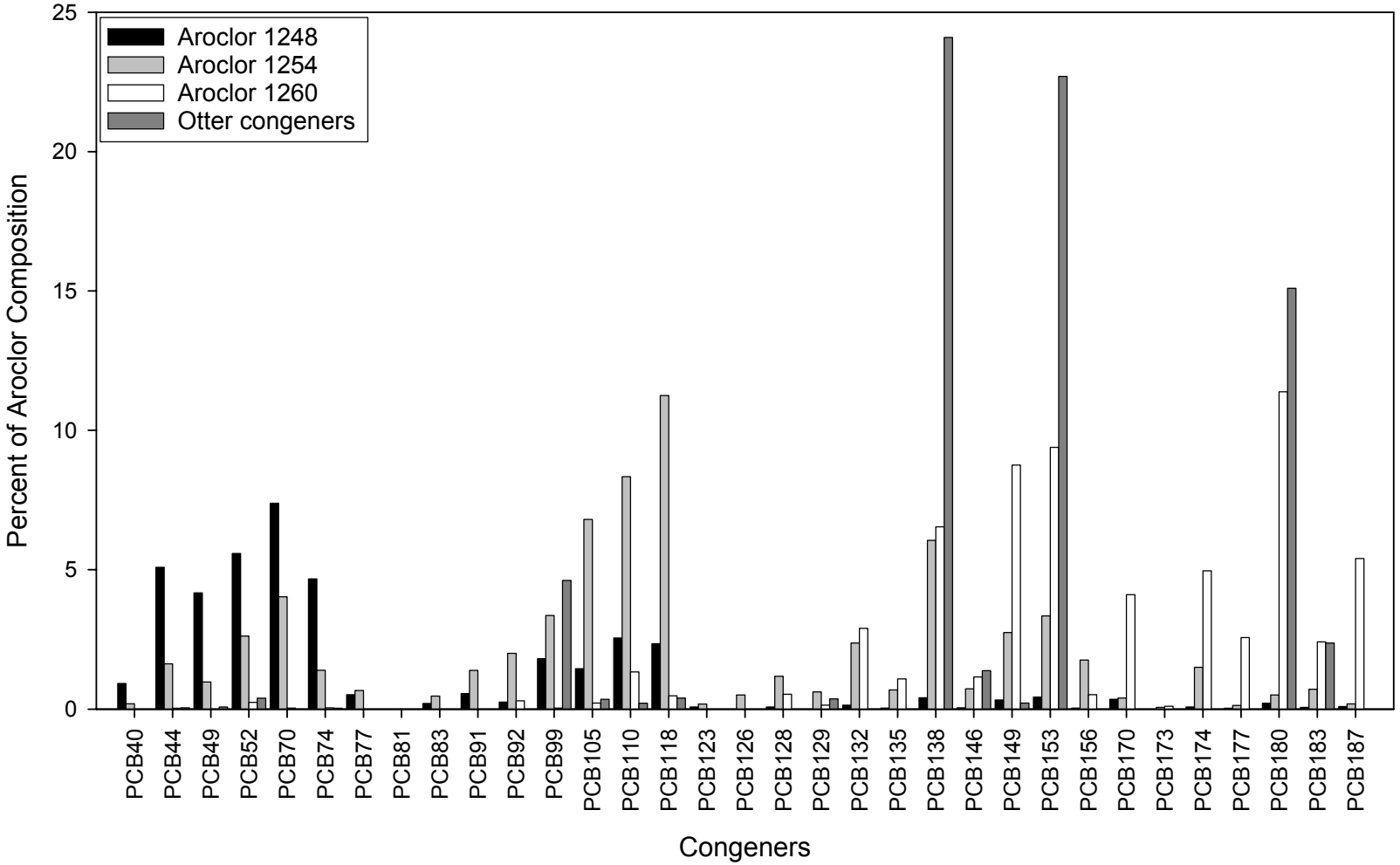


Figure 48. Comparison between congener profiles of Aroclors 1248, 1254, 1260 and adult male river otter PCB congener profile from liver tissue analyzed in this study. Aroclor congener data taken from Frame et al. (1996) and Kodavanti et al. (2001).

and mean liver congener composition of adult river otters from this study. Therefore, caution must be used when comparing PCB tissue concentrations data presented in the literature.

In general, the mean ΣPCB liver concentration for river otters collected from the LCR during this study was 16-fold lower (0.45 μg g^{-1}, n = 32) than otters collected in the same area in 1978-79 (6.99 μg g^{-1}, n = 7)(Table 26), though data reported by Henny et al. (1981) was based on Aroclors 1254/1260. Mean ΣPCB from the LCR was also lower than concentrations reported (Elliott et al. 1999) for otters collected from the same river segment (0.76 μg g^{-1}, n = 4) two years earlier. The data shows a continued decline in PCBs overall for the LCR, though localized heavily contaminated areas are still present (Portland Harbor, Bonneville Dam). Hepatic means for ΣPCBs from OC, SW, and OP were similar to but generally lower than means reported by Elliott et al. (1999) and Harding et al. (1999) for the relatively pristine upper Columbia, Fraser and Kootenay Rivers of British Columbia, Canada.

Recently, Kannan et al. (2000) calculated a no observed adverse effect level (NOAEL) and a lowest observed adverse effect level (LOAEL) threshold value for ΣPCBs based on hepatic tissue concentrations from the Eurasian otter at 170 ng g^{-1} and 460 ng g^{-1}, respectively. All regional location ΣPCB means for juvenile males from this study were below the LOAEL established by Kannan et al. (2000), with means from the LCR (241 ng g^{-1}) and WB (217 ng g^{-1}) the only locations above the NOAEL (Table 10). For adult males, ΣPCB mean concentrations for locations LCR (564 ng g^{-1}), PS (585 ng g^{-1}), WB (219 ng g^{-1}) and WW (322 ng g^{-1}) were above the NOAEL, with means from the LCR and PS above the LOAEL. Overall, ΣPCB means for juvenile (89.3 ng g^{-1}) and adult (107 ng g^{-1}) males were at or below the NOAEL. In general, most of the 196 otters analyzed for PCBs from Oregon and Washington were below both the NOAEL and LOAEL, with individual otters (2 juveniles, 11 yearlings, 18 adults) from the more contaminated locations exceeding the LOAEL threshold level. Except for the few individuals mentioned above, the majority of otters should

exhibit no adverse effects. The Eurasian otter is generally thought to be more sensitive to PCBs than the river otter from North America, though no comparative data exists to support this conclusion. If true, then threshold toxicity values developed for the Eurasian otter would be conservative in their use to evaluate potential PCB effects on the river otter in this study.

The river otter PCB congener profile (all ages combined) was predominated by the higher chlorinated congeners 99, 138, 153, 170/190, 180, and 182/187, which constituted 79% of the ΣPCBs analyzed. The congener pattern with PCB 138 as the dominant congener was the same for both age groups from the seven regional locations sampled, except adult males collected from the PS with PCB 153 the dominant congener (Figures 28 and 29). The congener pattern of the PS adult males is similar to that of cetaceans and sea otters from the North Pacific and Asian coastal waters (Minh et al. 2000, Ross et al. 2000, Kannan et al. 2004) and the Eurasian otter (Kruuk and Conroy 1996, Tans et al 1996, Smit et al. 1998). The difference for the PS otters may be due to dietary differences; most adult males from the PS were trapped at or near the marine environment.

Non-ortho Substituted PCBs, Dioxins and Furans

A paucity of published non-ortho PCB, PCDD and PCDF data exists for both mink and otter. Data from this study for the LCR are comparable to data presented in Elliott et al. (1999) for non-ortho PCBs, dioxins and furans for otters collected from the same area in 1990-91 and 1991-92. No decrease was apparent in these contaminants. Non-ortho PCB, PCDD and PCDF otter concentrations from this study were also comparable to data collected from otters trapped from the Columbia, Fraser and Kootenay Rivers of British Columbia, Canada in 1994-95 and 1995-96 (Harding et al. 1999). Non-ortho PCBs, PCDDs and PCDFs were also comparable to mink data from the Saint Maurice River in Quebec, Canada in 1991 (Champoux 1996) and mink from the

Acadia Parish in southern Louisiana in 1999 and from southern South Carolina in 2000 (Tansy et al. 2003).

Two-thirds of the otters with individual TCDD concentrations above 1 ng kg^{-1} were collected near pulp and paper mills previously using elemental chlorine in the pulp bleaching process. These otters also had quantifiable concentrations of total H6CDD, H7CDD and OCDD. Only one of the 12 otters with hepatic TCDF concentrations of above 1 ng kg^{-1} was associated with pulp and paper mills described above. The remaining otters with TCDD and TCDF above 1 ng kg^{-1} were mostly located along the coast and away from industry, suggesting non-point sources resulting from atmospheric deposition (industry, forest fires, etc.). Otters with significant hepatic concentrations of total H6CDD, total H7CDD and OCDD may result from the use of PCP as a wood preservative in which higher chlorinated dioxins and furans are impurities at μg g^{-1} concentrations (Schwetz et al. 1974, Goldstein et al. 1977, Elliott et al. 1999). The high TCDD (506 ng kg^{-1}) and OCDD (1521 ng kg^{-1}) concentrations for juvenile male RAG-204 trapped west of Chehalis, WA was unusually high and may have resulted from local exposure.

TEQs

Juvenile and adult total TEQs were similar for each location, with the LCR being higher than the other locations. Furthermore, no significant age related differences were found in total TEQs. Using the liver based NOAEL (42 ng kg^{-1}) and LOAEL (84 ng kg^{-1}) established by Kannan et al. (2000) for the Eurasian otter, the total TEQs by location and age for otter males in this study were all well below that of the NOAEL. Individually, only three juvenile, three yearling and two adult otters were above the NOAEL, with only the juvenile male RAG-204 collected near Chehalis and the yearling male RAG-025 collected in Portland Harbor near the Fremont Bridge had TEQs above the LOAEL. Based on TEQ thresholds developed by Kannan et al. (2000), no observable adverse effects on

otters in this study would be expected, except perhaps the two otters mentioned above.

Perfluorooctanesulfonate and related fluorinated compounds

Perfluorooctanesulfonate, a persistent acid, and other fluorinated compounds are widely distributed in human, wildlife and environmental media (Luebker et al. 2005b). The unique chemical properties of fluorinated surfactants, derivatized from perfluorooctanesulfonyl fluoride, have allowed their use in a wide variety of commercial products for over 50 years (Kannan et al. 2002, Olsen et al. 2003). The fluorinated organic compounds have been used for paper and packaging treatments and as surface protectants for carpet, fabrics, upholstery and leather, and in fire fighting foams. These sulfonated fluorochemicals can repel both water and oil, and reduce the surface tension to a much lower level than other hydrocarbon surfactants (Moody and Field 2000). Sulfonated fluorochemicals are also more stable, and function better in the environment where other compounds rapidly degrade. However, several of the fluorinated compounds (FOSA, PFOA, PFHxS, PFOS) began appearing up in human blood samples as early as 1968, though only identified as organic fluorine compounds (Giesy and Kannan 2001, Hansen et al. 2001, Olsen et al. 2005). Findings of fluorinated compounds in human sera reported over the next several decades suggested widespread distribution, both in human populations and the environment (Giesy and Kannan 2001). However, it was only the recent development of a compound-specific analytical method that allowed analyses of specific fluorochemicals in biological matrices with sufficient recovery and sensitivity (μg kg^{-1}) to measure environmentally relevant concentrations (Giesy and Kannan 2001, Hansen et al. 2001).

Little is known about the toxicity to humans and wildlife of PFOS, FOSA and other fluorinated chemicals. Related fluorochemicals have been shown to affect cell to cell communication, membrane transport, chemical energy production and increased peroxisome proliferation (Giesy and Kannan 2002).

Schnellmann and Manning (1990) reported FOSA to be one of the most potent uncouplers of oxidative phosphorylation. Recent research with rats demonstrated neonatal mortality from *in utero* exposure to PFOS (Luebker et al. 2005a, Luebker et al. 2005b). Rat maternal liver concentrations at gestation day 21 (GD21) of PFOS from doses of 0.1, 0.4, 1.6 and 3.2 mg/kg/day were 20.9, 82.5, 233 and 517 μg g^{-1} (ww), respectively. Maternal transfer of PFOS was also documented, with rat fetus liver concentrations at GD21 of 7.92, 30.6, 86.5, 230 μg g^{-1} (ww) for the same maternal dosing (Luebker et al. 2005b). By lactation day 5 (LD5) rat maternal liver concentrations of PFOS were 47.9, 110 and 146 μg g^{-1} (ww), while rat pup liver concentrations were 73.4, 274 and 245 μg g^{-1} (ww), respectively with PFOS doses of 0.4, 1.6 and 2.0 mg/kg/day. Reproductive outcome was affected at the highest dose (3.2 mg/kg/day PFOS) by decreasing the number of uterine implantation sites and reducing the gestation period, increased number of dams with stillborn pups or with all pups dying by LD4 (Luebker et al. 2005a). Toxicity is thought to occur at the level of the mitochondria by interfering with mitochondrial metabolic pathways, inducing peroxisome proliferation (Starkov and Wallace 2002). Perfluorooctanyl compounds are thought to disrupt mitochondrial function by three mechanisms: protonophoric uncoupling of mitochondrial respiration, induction of the mitochondrial permeability transition and nonselective increase in membrane permeability (O'Brien and Wallace 2004). The sequence of events was found to be initiated by induction of the mitochondrial permeability transition, which resulted in the release of cytochrome *c* as well as other cofactors, thus leading to the inhibition of mitochondrial respiration and the resultant generation of reactive oxygen species (O'Brien and Wallace 2004). Therefore, the toxicity to perfluorooctanyl compounds is reflected by mitochondrial respiratory dysfunction, which is exacerbated by the resulting oxidative stress and damage (O'Brien and Wallace 2004).

Concentrations of PFOS and FOSA in livers of river otters collected from Oregon and Washington were lowest along the coastline, but higher from

urbanized waterways. The largest PFOS concentration (994 ng g^{-1}, ww) was from an adult male otter collected on the Willamette River near Albany, Oregon, with the highest FOSA concentration (71.6 ng g^{-1}, ww) from a PS adult male collected near Fort Ward, Washington. The ratio of mean FOSA to PFOS was different among the three regions (PS, W, OC + OP), with the concentration of FOSA 21.0, 3.60 and 9.55% of PFOS in river otter livers. This may be due to location specific fluorochemical source differences (Kannan et al. 2002). River otters from Oregon and Washington have the highest reported PFOS concentrations reported for aquatic mammals from the west coast of the United States, suggesting greater exposure potential to perfluorochemicals from the more urbanized inland waterways than the more sparsely populated coastal and marine environs of the west coast (Kannan et al. 2001, Kannan et al. 2002). It appears that perfluorochemical contaminants (especially PFOS and FOSA) are widespread; they were found at all locations sampled. The lack of age or sex related differences in perfluorochemical concentrations may be due to their unique hydrophobic and oleophobic properties (Moody and Field 2000, Kannan et al. 2002). Perfluorochemical concentrations were substantially lower in river otter livers collected in this study than reported to cause reproductive problems in rats (Luebker et al. 2005a, Luebker et al. 2005b). Further investigation is needed to evaluate perfluorochemical toxicity to aquatic mammals using environmentally relevant concentrations.

Using Scats as a Monitoring Tool

Regional/drainage scat samples from Eurasian otters have been used to examine OC and PCB contaminant exposure in relationship to otter population status (Mason et al. 1992, Mason 1993, Mason and Macdonald 1993, Gutleb and Kranz 1998). Scats have been also used to estimate Eurasian otter daily intake and potential body burdens (Mason et al. 1992), though no direct comparisons were made between scat and tissue contaminant concentrations. Larsson et al. (1990) reported that OCs and PCBs in Eurasian otter scats come

from two sources: OCs and PCBs that are not absorbed by the digestive tract and from anal gland secretion which may be rich in lipids. In a feeding study, Eurasian otters assimilated about 91.6% of ingested PCBs (Smit 1990). Remaining PCBs passed through the digestive tract and were excreted in scats. Some authors suggest that the addition of OC contaminants via the anal gland is small relative to the dietary contribution (Mason and Macdonald 1994). Depending on the type and extent of OC and PCB exposure, daily intake estimates can be made to determine potential body burden accumulation over time (Mason et al. 1992). Calculating the potential body burden, however, does not consider the otter's ability to metabolize OCs and PCBs, either by rate of metabolism or by the ability to metabolize specific OCs or PCB congeners. If contaminants in the otter diet were a result of continuous and consistent exposure, the scat contaminant profile should reflect the contaminant profile of the body except for small differences in those congeners which can be metabolized. But, if the majority of contamination exposure originated from localized contaminant point sources of differing profiles and concentrations, scat contaminant profiles would less likely reflect contaminant body burdens. Therefore, caution must be used when trying to estimate otter contaminant daily intake and potential body burden as they may not reflect actual contaminant concentrations. What stands out from these studies though, is the ability to examine OC and PCB exposure based on scat contaminant concentrations. As otters occupy and maintain discrete territories, scat samples collected at latrine sites could point to localized pollution point sources. Using otter scats to monitor contaminants within a river system or drainage could aid in the location and remediation of currently unknown contaminant point sources.

Otter Biology and Relationships With Contaminants

The 303 river otters collected during this study were generally in good condition. Observed fat deposition was consistent with that reported by Baitchman and Kollias (2001), though it was further noted that fat deposition

extended anteriorly along the spine and over the abdominal muscles as otters gained body fat. The two yearling males (the age class that disperses) considered very thin (OT-04, RAG-074) had no observed gross pathologies and low liver contaminant concentrations. Their condition may have resulted from their inability to find a suitable territory, which could result in a poor diet.

Juvenile and possibly yearling otters with baculum breaks shown in Figure 9 had DDE, dieldrin and ΣPCB concentrations below 200 μg kg^{-1} except for otter RAG98-04 which had 425 μg kg^{-1} ΣPCBs. A Chi-square test showed baculum fractures were age independent, suggesting that baculum fractures occurred during the first and possibly second year of life when baculums are small and fragile. Overall, 9.9% of the baculums from this study had been fractured, which seems like a high percentage for a highly evolved aquatic mammal (see later discussion of baculum length and mass associated with PCBs). An incidence of river otter baculum fractures was reported by Friley (1949), who noted 4 (2 juvenile and 2 adult) of 86 baculums collected from the upper and lower peninsulas of Michigan had been previously fractured (4.7%). A Chi-square test on the incidence of baculum fractures between data from Friley (1949) and this study resulted in a Chi-square value of 2.2193 and P = 0.1363.

The renal and adrenal agenesis of OT-038 and the apparent lack of external or internal testes (cryptorchidism) of OT-036 at river mile 119.5 of the LCR was associated with some of the highest ΣPCB (2120 and 926 μg kg^{-1}, respectively) and DDE (1821 and 1036 μg kg^{-1}, respectively) concentrations found for otters from Oregon and Washington. However, Harding (2002) noted a yearling male otter having a left undescended testis and renal agenesis on the same side and a three year- old male with only one testis which was abnormally small. Both otters had liver DDE and PCB concentrations below the detection limit of 0.002 μg g^{-1} (ww). The yearling otter (RAG99-01) trapped from Woahink Lake south of Florence, OR in this study which had suspected Müllerian tissue associated with epididymal tissue and no prostate tissue associated with the prostate section of the urethra is another example. This male otter came from a

fairly pristine location with very low contaminant concentrations in the liver, yet had several reproductive organ anomalies. Therefore, care must be taken in associating pathological findings with contaminant concentrations as the incidence of adrenal, gonad and renal agenesis and reproductive organ deformities in uncontaminated otter populations is poorly understood.

The two females exhibiting the perineal abscess and vaginal infection may have been exacerbated by high liver ΣPCB concentrations (1640 and 2400 μg kg^{-1}), which may have compromised their immune system. Otters collected from the lower Willamette River, north of Redmond, WA, Wenatchee, WA and from the Bremerton area of the PS also had contaminant concentrations as high or higher, but exhibited no gross pathological findings.

Little or no published histological information is available on river otters to compare with data presented here. The 41% incidence of pulmonary findings (i.e., pulmonary granulomas, diffuse pulmonary congestion, edema) was not unexpected due to the aquatic nature of the species and the potential for water aspiration. The pulmonary findings were not dependent on location, suggesting contaminants from the higher polluted LCR and WB were not the cause. Special staining of the kidney tissue from juvenile male otter RAG-010 was strongly suggestive of leptospirosis, making this the first reported case for the species. The juvenile female (RAG99-02) exhibiting the vaginal infection from the blackberry stem was severe enough to limit its mobility, possibly compromising its ability to hunt for food which was reflected in its blood chemistry. The epididymitis found in male otters did have significant dependence to location, which was more prevalent for male otters from LCR and WB with higher contaminant concentrations than males from the coastal areas. Hepatic mean concentrations of DDE, ΣPCBs and OCDD for LCR and WB otters combined were 549, 689 and 386 μg kg^{-1} (ww, n = 5), while means for coastal males were 2.20, 97.7 and 30.1 μg kg^{-1} (ww, n = 3), respectively. The epididymitis may be associated with these contaminants and/or other contaminants.

Yearling male otter RAG99-03 from the Willamette River near the Fremont Bridge in downtown Portland, OR did have active spermatogenesis, but with a few spermatid giant cells in the spermatic tubules and the epididymis containing only degenerate epithelial cells. The yearling male also had high concentrations of OCs, PCBs, PCDDs and PCDFs (904, 2407 ng g^{-1} DDE and ΣPCBs and 3429 and 954 ng kg^{-1} OCDD and total H7CDD).

Histologic evaluation of the male reproductive system found that none of the 64 juvenile male otters examined exhibited seasonal gonadal hypertrophy or spermatogenesis. These findings confirm observations by Liers (1951) and Hamilton and Eadie (1964) that 8 to 12 month old juvenile male otters are still prepubertal, unable to produce spermatozoa. Testicular T production was also absent in juvenile males, further supporting evidence that otter males less than one year old are still prepubertal. Yearling otters with paired testes mass above 11 g demonstrated spermatogenesis, which is considerably less than the arithmetic mean of 28 g reported by Hamilton and Eadie (1964). In fact, all but 2 of the 27 yearling otters producing spermatozoa had a testes mass less than the mean 28 g reported by Hamilton and Eadie (1964). Spermatozoa were seen in yearling testes as early as the first week of December, while spermatozoa in adult testes were seen as early as mid-November.

Organ mass and contaminants showed several significant relationships for both juvenile and adult male otters, though the adjusted r^2 values were generally low based on simple regressions (Tables 24 and 25). Using body length as a second variable (which adjusts for actual age in growing juvenile animals) eliminated many of the significant relationships initially observed with simple regressions. Some of the remaining relationships included mercury concentrations in livers inversely associated with liver and kidney masses for juvenile males and also liver, spleen, pancreas and adrenal masses for adult males. However, the mercury relationships were the strongest with liver and kidney masses for juvenile males (adjusted r^2 above 0.30) (Tables 24 and 25). Overt liver and kidney toxicity in wildlife and domestic animals is usually

associated with organ enlargement and necrosis (Pathak and Bhowmik 1998, Henny et al. 2002). The correlation matrix table in Appendix I was examined to see if any highly significant correlations existed between Hg and other metals or halogenated hydrocarbons which might explain the inverse relationship between Hg and liver and kidney mass. No highly correlated relationships ($P < 0.0001$, $r > 0.50$) between Hg and other metals or OCs, PCBs, PCDDs and PCDFs were found. Manganese was the only metal with an r value (positive) above 0.45. Mercury was also positively correlated with Ca ($r = 0.299$), Mg ($r = 0.263$) and Zn ($r = 0.330$). None of the significant correlations between Hg and the OCS, PCBs, PCDDs and PCDFs had r values above 0.45 and all correlations were negative. No explanation can be offered for the inverse relationships between Hg and liver and kidney masses or the other tissues mentioned above.

Liver concentrations of HCB also had inverse relationships with both liver and kidney masses in juvenile, but not adult otter males. Hexachlorobenzene toxicity is usually associated with porphyria, though continued exposure to toxic concentrations of HCB can cause further liver damage. However, HCB toxicity to otters is not expected to occur at liver concentrations found in this study. There were no significant correlations with HCB and any metals, but highly significant correlations were found with HCB and most of the OCS and PCBs, but no correlations with PCDDs or PCDFs. It is unclear why HCB was negatively correlated with both juvenile liver and kidney masses.

The inverse relationships between thymus mass and hepatic concentrations of chlordanes, dioxins, HCB and PCBs (Tables 24 and 25) were similar to findings reported in the literature, i.e., exposure results in thymic atrophy (Mason et al. 1986c, Bondy et al. 2003, Laiosa et al. 2003, Tan et al. 2003, Tan et al. 2004, Beineke et al. 2005). Multiple regression analyses between river otter thymus mass and PCBs after adjusting for age in years (Table 26), showed the majority of significant relationships were with the Class A *di-* to *tetra-ortho* chlorine substituted PCB congeners having no vicinal hydrogen atoms (Figure 38). These PCB congeners are the most difficult to metabolize

and comprise 48 percent of the ΣPCB concentration (Kannan et al. 1995, Boon et al. 1997, Leonards et al. 1998, Kucklick et al. 2002, Li et al. 2003). Class B PCB congeners (41% of the ΣPCBs analyzed) were next in the number of significant relationships with thymus mass, which for river otters, also appear to be fairly difficult to metabolize. The PCBs most readily metabolized are AhR mediated non-ortho substituted congeners having vicinal hydrogens in the *ortho-meta* positions (i.e., PCBs 15, 77, 78, 81), and are the preferred substrates for CYP1A1 (Li et al. 2003). These PCBs are rarely seen in animal tissues. Class D PCB congeners are next in ease of metabolism as they contain both *meta-para* and *ortho-meta* vicinal hydrogens, which are substrates for both CYP1A and 2B isoenzymes. Class D PCB congeners had only one significant relationship with PCB 87. Class C PCB congeners having vicinal hydrogens in the *meta-para* position had no significant relationships to thymus mass. The lack of significance may be due to the small concentrations of PCBs in Classes C and D that are more readily metabolized compared to the other congener classes. Data for river otters presented in Table 26 agrees with published findings of Blankenship et al. (2005) concerning PCB biomagnification based on structural similarities of vicinal hydrogens and ability for metabolism. Otter findings from this study (Table 26) also generally agrees with published findings for several species of pinnipeds and cetaceans, the Eurasian otter and polar bear (Ursus maritimus) concerning PCB congener concentrations using the class system (Boon et al. 1997, Kucklick et al. 2002, Li et al. 2003, Chou et al. 2004, Metcalfe et al. 2004).

Results in recent publications suggests that the Class A *ortho*-substituted PCBs mentioned above destroy thymus cells readily dissolve into membranes, disrupting membrane structure, with little or no membrane disruption occurring from non-*ortho*-substituted PCBs (Tan et al. 2004, Yilmaz et al. 2006). *Ortho*-substituted PCB membrane alteration is thought to increase membrane fluidity, subsequently altering membrane protein function. It appears that the bulky three dimensional structure of *ortho*-substituted PCBs is enough to perturb membrane

lipids, thus altering membrane fluidity. This has been demonstrated in endoplasmic reticulum, mitochondrial and plasma membranes. Of the two cell types studied (thymocytes and cerebellar granule cell neurons), *ortho*-substituted PCB membrane disruption was sufficient to cause cell death within a brief period of time. PCB altered membrane fluidity was also documented in cultured rat renal tubular cells (Lopez-Aparicio et al. 1997).

Contaminant Relationships With The Male Reproductive System

In this field study, it was important to understand the reproductive status of juvenile male river otters in order to possibly identify reproductive effects associated with contaminant exposure. The finding that juvenile males are prepubertal during their first year of life eliminated the need to understand or explain potential confounding issues of androgen based pubertal growth and masculinization of the male reproductive system, as well as seasonal reproductive organ hypertrophy. The actual animals age remained a confounding issue when estimating male reproductive system growth and development for each individual juvenile male. Birth dates were not known for any of the individual otters at the time of capture and the capture date provided only a rough approximation of age. Therefore, use of capture date to account for age differences was not an adequate measure to use in the statistical model. Instead of using age, individual juvenile body length was used to account for size differences and estimating age in the statistical model used to associate contaminant concentrations with several reproductive parameters.

No obvious differences in reproductive tract morphology were found (except for OT-36 which had no apparent internal/external testes) between otters from areas of low contaminant concentrations versus those areas with high concentrations, which suggests that the sensitive period of androgen controlled virilization of the embryonic male otter reproductive tract was not significantly affected by *in utero* contaminant exposure. Animal dosing studies have found no morphological abnormalities of the male reproductive tract as a result of OC,

PCB, PCDD or PCDF exposure *in utero*, except for anogenital distance in rats and mice which decreases with increasing DDE, PCB and TCDD concentrations (Malby et al. 1992a, Gray et al. 1995, Faqi et al. 1998a, You et al. 1998, Wolf et al. 1999). Dosages used in these studies were also considerably higher than environmental exposure. In addition to TCDD and PCB congeners which have the most significant effect on anogenital distance, non-ortho substituted forms (i.e., PCB 77, PCB 126, PCB 169) also interact with the AhR. A recent study examined men from central Taiwan who were transplacentally exposed to PCBs in 1978-79 from maternal consumption of PCB contaminated rice oil (Guo et al. 2000). External genitalia of the exposed men appeared normal compared to controls, however, abnormal sperm morphology and motility were observed. This is similar to the findings of yearling male river otter RAG99-03 collected from the Willamette River in downtown Portland which had active spermatogenesis, yet had abnormal sperm morphology and degenerate epithelial cells of the epididymis.

The significant inverse relationships found in this study between liver contaminant concentrations (mainly *ortho*-substituted PCBs) and juvenile male otter gonad mass, testicle mass, baculum length and mass and prostate mass (Table 27) support preliminary findings reported by Henny et al. (1996), where PCBs provided the strongest inverse relationships. DDE was also inversely related to juvenile male baculum length and mass. Equally important was the finding in this study that significant inverse relationships existed between several PCBs and OCs and adult male otter baculum length and baculum mass (Table 28). The inverse relationships found with adult males suggests the reproductive organ hypoplasia observed in juvenile male otters was not the result of delayed development, but was a permanent effect. Decreasing male reproductive organ mass and size associated with increasing contaminant concentrations was not overtly conspicuous, but subtle, except at the highest contaminant concentrations. Could reduced baculum length and mass (i.e., strength) be

responsible for the 9.9% incidence of broken baculums in the Oregon and Washington study area?

Juvenile male river otters in this study were exposed to environmental contaminants through both *in utero* and lactational routes, with continued contaminant exposure occurring into and throughout adulthood via their diet. The extent of contaminant contribution by transplacental and lactational routes is unknown for otters. In rats, offspring received more of the maternally dosed lipophilic compounds (dioxins and coplanar PCBs) through lactation (7-28%) than transplacentally (0.5-3%) (Chen et al. 2001). You et al. (1999) reported rat pup liver DDE concentrations at PND10 were 50 times higher for the lactationally exposed group than the *in utero* exposed group. Nishimura et al. (2005b) also reported that lactational TCDD exposure of young rats was greater than *in utero* exposure. Prenatal contribution of PCB126 and PCB153 in juvenile goat body burdens at nine months of age was a much lower fraction compared to the lactational contribution (Lyche et al. 2004, Oskam et al. 2005). The greater demands of lactation mobilizes lipophilic contaminants like PCB 153 from maternal body reserves during milk production, exposing nursing offspring to greater contaminant concentrations. Thus, tissue concentrations of *in utero* and lactationally exposed offspring are far greater than those exposed exclusively through the placental route (You et al. 1999).

Research in this dissertation was not designed to elucidate possible mechanism(s) responsible for the observed male river otter reproductive organ hyperplasia. In recent years, however, many experimental dosing studies have been published (mainly with the rat model) concerning the effects of polyhalogenated hydrocarbon exposure on the developing male reproductive system. To date, no mechanism(s) have been elucidated pertaining to observed endocrine disruption as a result of prenatal and/or postnatal OC, PCB, PCDD or PCDF exposure. A review of the literature was conducted to examine currently proposed pathways and mechanisms of endocrine disruption which may apply to the male river otter reproductive organ hypoplasia observed in this study. And as

juvenile male river otters were maternally exposed both *in utero* and lactationally, studies examined were restricted to the same dosing regimes, with other than maternal postnatal dosing studies used only as supplemental information.

Due to their abundance in developing testes, the Sertoli cell population dictates final adult testes size, and factors affecting the number of Sertoli cells produced will have effects on both final testicular cell volume and mass (Jannini et al. 1995, Buzzard et al. 2000). The primary mitogenic hormone responsible for inducing Sertoli cell proliferation is FSH, with T and *β*-endorphin (produced by Leydig cells) used as negative hormone regulators of proliferation (Bardin et al. 1994, Sharpe 1994). Sertoli cell proliferation occurs only during late gestation and early neonatal periods (Buzzard et al. 2000). Each Sertoli cell is capable of supporting only a finite number of germ cells to maturity, and it is the final number of Sertoli cells produced which determines final spermatogenic potential of the adult testes (Buzzard et al. 2000). What stops Sertoli cell proliferation when reaching adult size is still unknown. However, those hormonal factors controlling the rate and duration of Sertoli cell proliferation ultimately control reproductive fertility. Recent research suggests that the thyroid hormone triiodothyronine (T_3) is integrally involved in regulating final testicular size and Sertoli cell differentiation (Jannini et al. 1995, Brouwer et al. 1998, Stoker et al. 2000). It is known that (T_3) binds with high affinity and low capacity to the nuclei of immature Sertoli cells. The concentration of Sertoli cell nuclear thyroid hormone binding sites changes during gonadal development. Maximum expression of thyroid binding sites occurs from late gestation into early neonatal life, decreasing throughout prepubertal growth, with little or no expression in the adult (Jannini et al. 1995). Germ and interstitial cells have little T_3 binding activity, confirming that the major target of thyroid hormone in the testes are the somatic cells of the seminiferous epithelium. Fetal and neonatal hypothyroidism in rats leads to prolongation of Sertoli cell proliferation, increasing testes size by as much as 62% and an 84% increase in Sertoli cell numbers (Van Haaster et al. 1992, Hess et al. 1993, Simorangkir et al. 1995). Hyperthyroidism has the

reverse effect by reducing the Sertoli cell proliferative period and accelerating their differentiation into functional non-proliferating secretory cells (Van Haaster et al. 1993, Cooke et al. 1994). Gonocyte numbers increase at this time as well, but as an indirect effect of excess T_3 secretion on the Sertoli cells because the gonocytes do not express functioning thyroid hormone receptors (TR). Early differentiated secretory Sertoli cells begin expressing nutrients (lactate) and growth factors like insulin-like growth factor-I (IGF-I) which stimulates mitotic germ cell DNA synthesis via paracrine signaling (Cooke et al. 1994, Jannini et al. 1995).

One of the most consistent effects resulting from PCDD and PCB exposure in all age groups of mammal species (including humans) studied to date, is a decrease in serum thyroxine (T_4) concentrations, both bound and free (Van Birgelen et al. 1994, Mckinney and Waller 1994, Morse et al. 1995, Osius et al. 1999, Skaare et al. 2001, Ulbrich and Stahlmann 2004, Debier et al. 2005). Polychlorinated dibenzo-*p*-dioxin and PCB related decreases in thyroid hormone are thought to occur by either: a) interference with thyroid function and regulation, b) induction of thyroid hormone metabolism, c) competitive binding with thyroid hormone transport binding proteins or some combination of the three (Brouwer et al. 1998). *In utero* PCB and TCDD exposure was shown to alter thyroid gland morphology and function in rat offspring (Collins et al. 1977, Collins and Capen 1980, Sepkovic and Byrne 1984, Ness et al. 1993, Nishimura et al. 2005b). Increased biliary secretion and hepatic-peripheral elimination of T_4 followed TCDD and PCB exposure in rats (fetal, neonatal and adults), which was associated with increased T_4 deiodination and glucuronidation (Morse et al. 1993, Morse et al. 1996, Kato et al. 2003, Nishimura et al. 2005a). Thyroid hormone disruption is proposed to occur via the AhR (Kuriyama et al. 2003), as TCDD exposure in normal AhR mice reduced T_4 serum concentrations but not in AhR null mice (Nishimura et al. 2005a). Induction of UDP-glucuronosyltransferase (UGT) and P450s CYP1A1 and CYP1A2 (regulated by AhR) also did not occur in AhR null mice. However, the AhR is induced very little

with non-*ortho* substituted PCBs like congeners 118 and 153, but which also depress serum T_4 concentrations. Kato et al. (2003) showed a decrease in serum T_4 concentration after exposure to a PCB Kanechlor-500 mixture without induction hepatic UGT1A1 or UGT1A6, responsible for T_4 glucuronidation. Polychlorinated biphenyl congeners have been shown in rats and humans to bind to transthyretin with affinities similar to that of the natural ligand (Ulbrich and Stahlmann 2004), allowing for greater T_4 clearance. In contrast, PCB binding to thyroid binding globulin is very weak or not at all.

In utero-lactational and/or lactational contaminant exposure is important as it occurs during critical windows of embryonic cellular virilization, differentiation, and proliferation in the male reproductive system (Birnbaum 1994). Current research shows different responses between lactational and *in utero*-lactational PCB exposure in male reproductive organ development. Lactational and inter-peritoneal PCB exposure of neonatal rats decreased thyroid follicle size, colloid density and serum thyroxine (T_4) concentrations without changing serum T_3 or thyroid stimulating hormone (TSH) (Gray et al. 1993, Chu et al. 1994, Chu et al. 1996a, Chu et al. 1996b, Cooke et al. 1996, Kim et al. 2001, Khan et al. 2002), and with no significant changes in thyroid gland size (Kaya et al. 2002). However, lactational PCB exposure of young males increased Sertoli cell proliferation, testes mass and sperm production at adulthood (Sager 1983, Sager et al. 1991, Gray et al. 1993, Chu et al. 1996a, Cooke et al. 1996, Kim et al. 2001). It appears that “transient lactational PCB exposure” (usually single exposure dosing) induces hypothyroidism which releases thyroid hormone control of Sertoli cell proliferation, causing testes enlargement (Cooke et al. 1994, Cooke et al. 1996). Thyroid hormone replacement was found to decrease or eliminate the observed Sertoli cellular proliferation of PCB exposed neonates. It is proposed that thyroid hormones promote cellular differentiation of Sertoli cells in neonates from mitotic to nonmitotic cells, with the concomitant start of secretory function (Cooke et al. 1994).

In utero-lactational exposure to TCDD, non-*ortho* substituted and *ortho*-substituted PCBs had the same negative effects on thyroid hormone homeostasis, but there was also a reduction in testes size and daily sperm production in guinea pigs, goats and rats (Lundkvist 1990, Malby et al. 1992a, Malby et al. 1992b, Faqi et al. 1998b, Hany et al. 1999, Kaya et al. 2002, Kuriyama and Chahoud 2004, Oskam et al. 2005). It may be that the period of PCB exposure is long enough to be considered chronic, in which there is a cessation of Sertoli cell proliferative activity (Jannini et al. 1995). The absence of T_3 retards Sertoli cell maturation by delaying the appearance of the tubular lumen, resulting in abnormal Sertoli support of germ cell development, cellular degeneration and atrophy which reduces testes size. However, Kuriyama et al. (2003) found low doses of PCB 118 in rats permanently disrupted the hypothalamus-pituitary-thyroid axis, resulting in a significant increase in juvenile thyroxine concentrations. This proposed “thyroid resistance syndrome” resulted in smaller testes, epididymides, seminal vesicles and impairment of daily sperm production due to mild hyperthyroidism (Kuriyama and Chahoud 2004) which corresponds to findings of Van Haaster et al. (1993) and Cooke et al. (1994).

Several studies have looked at developmental male reproductive effects of prenatal and/or postnatal OC, PCB, PCDD and PCDF exposure through potential alterations in serum androgen concentrations and androgen production in goats, guinea pigs, mice and rats. In general, serum T concentrations and testicular T production are unaffected in prenatal and postnatal offspring after TCDD, PCB Aroclor, reconstituted PCB mixture and individual PCB congener *in utero* and/or lactational exposure, except after adulthood or at extremely high doses (Lundkvist 1990, Sager et al. 1991, Malby et al. 1992a, Gray et al. 1993, Roman et al. 1995, Cooke et al. 1996, Theobald and Peterson 1997, Faqi et al. 1998a, Faqi et al. 1998b, Hany et al. 1999, Kuriyama and Chahoud 2004, Oskam et al. 2005). It has been proposed that observed reproductive effects of the testes, prostate and other accessory organs are not due to altered androgen concentrations or receptors, but possibly by growth factors and receptors that

regulate cellular proliferation and differentiation of the urogenital system which are downstream or independent of androgen receptor action (Gray et al. 1995, Roman et al. 1995, Roman et al. 1998). These systems may or may not require binding of androgen to mesenchymal or stromal androgen receptors to initiate paracrine signals by epithelium to initiate cellular proliferation, morphogenesis and differentiation as in the developing prostate, seminal vesicles and other accessory organs (Roman et al. 1998). As an example, Abbott and Birnbaum (1990) showed a single *in utero* TCDD dose in mice induced hyperplasia of embryonic ureter epithelium as a result of altered regulation of epidermal growth factor receptors.

Lastly, recent research has focused on the potential effects of PCB exposure on male reproductive system antioxidant system and possible association(s) with concurrent reduction in male reproductive organ size (Oskam et al. 2004, Sridhar et al. 2004, Venkataraman et al. 2004, Murugesan et al. 2005a, Murugesan et al. 2005b). Aroclor 1254 dosing resulted in significant decreases in testis, epididymis, ventral prostate and seminal vesicle mass in adult male rats. The PCB exposure also resulted in significantly decreased prostatic and testicular antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST) and glutathione reductase (GR), with a significant increase in lipid peroxidation, hydrogen peroxide and hydroxy radicals (Sridhar et al. 2004, Murugesan et al. 2005b). Individual PCB congener dosing in adult mice showed a significant increase in apoptotic Leydig cells possibly due to oxidative stress (Oskam et al. 2004). Though current work has been done exclusively with adult male rats, it is possible the observed oxidative stress found in the male reproductive system also occurs in younger males during the more sensitive periods of cellular proliferation and differentiation.

The lack internal/external testes noted in juvenile male otter OT-36 may have resulted from *in utero* contaminant exposure, as suggested by the high hepatic contaminant concentrations accumulated from its natal area. Recent

studies have shown that fetal testicular descent during sexual differentiation can be disrupted by hormonally active compounds (Sharpe 2001). Testicular descent occurs during fetal sexual differentiation in two phases (transabdominal and inguinoscrotal descent), which is hormonally controlled. The first or transabdominal phase of testicular descent is not mediated by androgens, but is controlled by insulin-like factor 3 (Insl3) or relaxin-like factor, which is synthesized by Leydig cells. Inguinoscrotal descent, however, is mediated by androgens. Pregnant rats exposed to estrogen-like endocrine disruptors or PCBs during the period of fetal male sexual differentiation results in inhibition of Insl3 expression and consequent failure of transabdominal testes descent (Emmen et al. 2000, Nef et al. 2000). Androgen receptor mutations, impaired androgen synthesis and antiandrogen exposure can impair prenatal inguinoscrotal descent (Hussmann and McPhaul 1991, Spencer et al. 1991, Nef et al. 2000). The resulting cryptorchidism usually results in infertility and an increased risk of testicular cancer (Hutson et al. 1994).

There is a paucity of published information concerning relationships between liver contaminant concentrations and baculum length and mass. Only one published study (Harding et al. 1999) observed an inverse relationship between juvenile mink baculum length and hepatic PCB concentrations ($n = 12$, $r^2 = 0.50$, $P = 0.033$), though sample size was small and PCB concentrations were based on Aroclor 1260 standards. Significant but inconsistent inverse correlations between total PCBs and baculum size were also observed in juvenile male mink collected in British Columbia, Canada during the winters of 1998-2002 (Unpublished Data, L.K. Wilson and J.E. Elliott, Canadian Wildlife Service, Delta, British Columbia). A recent study examined the dietary effects of Aroclor 1254 on baculum development in 12-week old male mink exposed for 20 weeks at 2 mg kg^{-1} in feed (Aulerich et al. 2000). Baculum length and mass were correlated ($r = 0.705$) for control male mink, but not for dosed males. However, no correlations were attempted between mink liver PCB concentrations and baculum length and mass. Mean baculum length and mass of dosed male

mink (43.4 mm and 0.20 g, respectively) were shorter and lighter than control males (44.4 mm and 0.22 g, respectively), though not significantly. The young male mink were not dosed prenatally or lactationally during the critical window of reproductive development, but later as juveniles growing to adulthood. The authors suggest that the weak correlation between baculum length and mass ($r = 0.074$) suggests possible subtle developmental alterations could be occurring (Aulerich et al. 2000). Besides the inverse relationships between baculum length and mass individual PCB congeners found in this study, there were also inverse relationships between hepatic DDE concentrations and baculum length and mass in both juvenile and adult otters. DDE was the most prevalent OC found in the livers of river otter males collected from Oregon and Washington, and DDE is known to be a potent androgen receptor antagonist (Kelce et al. 1995, You et al. 1998, Gray et al. 2001). Weak associations have even been found between serum DDE concentrations and bone mineral density in human males (Glynn et al. 2000).

The chondrogenesis and osteogenesis of the male river otter baculum may be similar to the rat where immature stromal cells of mesenchymal origin begin to differentiate into the os penis *in utero* towards the end of gestation (Glucksmann et al. 1976, Yoshida et al. 1980, Murakami 1986, Murakami and Mizuno 1986, Murakami et al. 1995, Izumi et al. 2000). There are critical androgen dependent periods for cellular differentiation and ossification of the rat os penis, which are vulnerable to antiandrogens (Moore et al. 2001). Recent studies have shown permanent os penis abnormalities in male rats exposed neonatally to antiandrogens (Moore et al. 2001, Goyal et al. 2004, Goyal et al. 2005). The os penis in rats is comprised of a distal and proximal segment. The distal segment develops as fibrocartilage by four to six weeks of age postnally, which is calcified by week eight and endochondrally ossified by week 12 (Murakami et al. 1995). The cells that differentiate into fibrocartilage have ARs and can form into fibrocartilage only in the presence of androgens (Murakami 1986). After fibrocartilage calcification, the endochondral ossification can take

place independently of androgen, though androgen can promote the process (Murakami et al. 1995). Though no published studies are available, it may be that PCBs and DDE interfere with the AR mediated development of the os penis, producing shorter and lighter baculums.

Population Implications of River Otter Male Reproductive Organ Hypoplasia

The reproductive organ hypoplasia observed in male river otters from this study was subtle, except in males from heavily polluted aquatic areas of western Oregon and Washington. It is not known to what extent (if any) the effect of smaller male reproductive organs has on the reproductive ability of adult males living in these contaminated areas. The 9.9% incidence of fractured baculums in otters from this study may be due to prepubertal contaminant exposure, resulting in smaller more fragile baculums. The effects of a fractured baculum (even though fused) on the reproductive success of the male is unknown. There is also some indication that the river otter immune system may be compromised to some extent as evidenced by contaminant related decrease in thymus mass and gross pathological findings in otters collected from heavily polluted aquatic environs (i.e., epididymitis, abscesses). The effects of contaminant exposure on the male otter reproductive and immune systems is probably localized, but far from being fully understood.

CONCLUSIONS

Exposure to EDCs like DDE, alkylphenol ethoxylates, metabolites of vinclozolin, PCBs and PCDDs results in a variety of adverse reproductive effects in wildlife. Alterations of wildlife reproductive organ differentiation, growth and function can result from perinatal and neonatal exposure to EDCs, including sex reversal in fish, small penises in alligators, ambiguous gonads in amphibians and altered sex behavior in birds. Results from the preliminary river otter study (Henny et al. 1996) correlated concentrations of known endocrine disrupting

contaminants with observed juvenile male reproductive organ hypoplasia along the LCR, which made it abundantly clear that additional research was needed to more fully understand the situation. The large number of male otters collected during this expanded study contained both high and low concentrations of contaminants, permitting a more rigorous statistical evaluation of male reproductive organ morphology in relation to endocrine disrupting contaminant exposure.

In general, non-reproductive morphometric differences were limited between male otters collected from Oregon and Washington ($H_0$1 passed), except for a few significantly heavier organ masses for adult males from Oregon (pancreas, adrenal glands) and Washington (thymus, lung, thyroid glands). Epididymis, gonad and testes masses were also heavier for Oregon males. This appeared to be due to yearling and adult male otters from Oregon coming into reproductive readiness approximately 2 weeks earlier than their more northern Washington counterparts. Because of the earlier breeding season in Oregon, juvenile males from Oregon appeared to be chronologically ahead of Washington juvenile males in sexual development.

Regional differences existed in river otter contaminant body burdens ($H_0$2 failed), with otters from heavily populated and industrialized regions in western Oregon and Washington having significantly higher hepatic concentrations of many contaminants. Also, otters from areas of intensive agriculture had significantly higher OC concentrations. Male hepatic mercury concentrations among regions were similar for both juveniles and adults, except for LCR otters, which were lower. Mean mercury liver concentrations, though elevated, were well below concentrations considered toxic.

Thymus mass was negatively correlated with increasing concentrations of select chlordanes, dioxins, HCB, and PCBs for both juvenile and adult male river otters, which is suggestive immunotoxicity, resulting in thymic atrophy. The most

significant inverse relationships with thymus mass were associated with the more persistent ortho-substituted PCB congeners. Recent research suggests that, after dissolving into the membrane, the three dimensional structure of these contaminants disrupt membrane structure by increasing membrane fluidity, thus altering membrane protein function; as reported for *ortho*-substituted PCBs. Ultimately, altered membrane fluidity was found to disrupt endoplasmic reticulum, mitochondrial, and plasma membrane functions sufficiently to cause cell death over a short period of time.

Juvenile male river otters were prepubertal as signified by quiescent testicular T synthesis and the absence of spermatogenesis ($H_0$3 passed for juveniles). However, T production was noted in yearling male otters with paired testes mass above 11 g and concurrent demonstration of spermatogenesis ($H_0$3 failed for yearlings). Spermatozoa were seen in the seminiferous tubules of yearling testes as early as the first week of December.

Testosterone mediated reproductive organ morphology appeared normal in all but one of the male river otters examined, suggesting *in utero* contaminant exposure was insufficient to alter embryonic sexual differentiation. No apparent internal or external testes were found for juvenile male otter OT-36, which also had the second highest juvenile hepatic concentrations of DDE and ΣPCBs. Similar reproductive tract abnormalities have been reported for otters in regions of low contamination, and the normal occurrence of such abnormalities is unknown for otters. However, the lack of internal or external testes may have also resulted from inhibition of testicular transabdominal and/or inguinal descent due to contaminant exposure of EDCs like PCB153 which has been reported to exhibit estrogenic properties, and is one of the primary PCB congeners found in otter livers.

Significant inverse relationships were found between hepatic EDC concentrations (mainly *ortho*-substituted PCBs and DDE) and juvenile male river

otter gonad mass, testes mass, prostate mass and baculum length and mass ($H_0$4 failed). More importantly, there were significant inverse relationships found between baculum size of adult male otters and several endocrine disrupting OCs and PCBs. EDC related reduction in baculum size of juvenile male otters appears to be carried into adulthood as a permanent effect. Though adult male otter testes and prostate size could not be correlated with EDCs due to seasonal reproductive organ hypertrophy, contaminant related reproductive organ hypoplasia may also occur in adult male soft tissues, as with reduced baculum size. The contaminant associated reduction in male reproductive organ size was also not overtly conspicuous, but subtle, except at the highest contaminant concentrations.

As the population of Sertoli cells dictates final adult testes size, then reduction in testes size denotes a decrease in Sertoli cell numbers. Individual Sertoli cells can support only a limited number of developing germ cells, and a decrease in Sertoli cell numbers would result in decreased sperm production. Thus, the number of Sertoli cells present at puberty dictates fertility. If the EDC related reduction in testes size found in juvenile male river otters is permanent, then fertility is potentially reduced as well. It may be that chronic PCB exposure interferes with thyroid mediated Sertoli cell proliferation long enough to cause cessation of Sertoli cell proliferative activity. It may also be that low doses of PCBs like PCB 118 permanently disrupt the hypothalamus-pituitary-thyroid axis, resulting in a significant increase in juvenile thyroxine concentrations. The proposed thyroid resistance syndrome mentioned above may result in smaller testes, epididymides and seminal vesicles, impairing daily sperm production due to mild hyperthyroidism.

The function of the baculum in mammalian reproductive morphology remains controversial as the development of the baculum among species is highly variable. The energetics of baculum development and maintenance, as

well as the potential for fracture and infection, suggests the baculum is adaptive. One hypothesis is that the baculum is used to induce ovulation. As the river otter is thought to be an induced ovulator, appropriate baculum size may be critical for successful ovulation and fertilization to occur. PCBs and DDE may interfere with AR mediated development of the os penis, producing shorter and lighter baculums. Therefore, EDC related reduction in baculum size noted in this study may negatively affect the reproductive capacity of adult males living in heavily contaminated environments. The 9.9% incidence of fractured baculums in otters from this study may also be due to prepubertal contaminant exposure, resulting in smaller more fragile baculums. The effects of smaller baculums and/or fractured baculums (even though fused) on the reproductive success of the male otter, however, is unknown.

The river otter was used as a sentinel species in this study, to evaluate potential biological effects resulting from environmental contaminant exposure. However, conclusions of cause and effect from such uncontrolled field studies are difficult, as information gathered provides little, if any, information on the mechanism(s) of action resulting from chemical(s) exposure. Evidence of EDC related male otter reproductive organ hypoplasia was documented in this study, and several potential mechanisms of endocrine disruption were discussed, which seem possible or even likely. But, elucidation of actual mechanisms causing the observed reproductive organ hypoplasia in male otters will not occur with further field work. The river otter is a good sentinel species for evaluating exposure, accumulation and possible effects resulting from environmentally relevant contaminants in field studies. But, it is a poor candidate for mechanistic laboratory studies due to its large size and low reproductive success in captivity. I believe the mink to be a better animal model than the rat or mouse in determining potential mechanisms responsible for the EDC related male reproductive organ hypoplasia observed in otters, as is a closely related species

of the mustelid family, sharing similar physiology, habitat use and diet. Mink breed readily in captivity and has been successfully used in several laboratory studies concerning potential effects of EDC exposure on female reproductive success and kit survival. However, little research has been done concerning potential EDC effects on the male mink reproductive system. Mink are also much more sensitive to a variety of environmental contaminants than otters, especially PCBs, PCDDs and PCDFs. Also, the inverse relationships between EDCs and male otter baculum size reported in this study have been observed in mink.

It is important to validate the EDC related male reproductive organ hypoplasia reported in this dissertation under controlled laboratory conditions, using environmentally relevant contaminant mixtures and concentrations based on the otter's diet. Contaminant mixture components (i.e., DDE, PCB congeners) could also be evaluated separately based on concentrations and endocrine disrupting potential. Contaminant exposure (dietary route) should start in reproductive mink females several months prior to breeding, throughout pregnancy and lactation, with dietary exposure of the offspring continued as they begin to eat solid food through to puberty. Controls and select dilutions of contaminant concentrations found in the diet would be used to evaluate dosage effects. Males should be sampled at select periods of age to determine general health, reproductive organ growth and development, hormone expression, tissue receptor number and contaminant body burdens. Age periods selected could include critical windows of proliferative and differential periods of development.

The body of evidence is growing, suggesting EDCs are affecting normal endocrine system function in wildlife and humans. Evidence comes from endocrine-related adverse effects observed in several wildlife species (amphibian, avian, fish, invertebrate, mammalian), increased incidences of endocrine-related human diseases and effects observed in animals exposed to

EDCs in controlled laboratory studies. Development of the mammalian male phenotype depends first on fetal testes formation, with subsequent androgen production by the fetal testes to drive the indifferent reproductive tract in becoming male. As the male fetus grows, other hormonal factors begin to modulate cellular differentiation and proliferation, providing important feedback regulation which directs development of the male internal and external reproductive tract, neural-endocrine development of the hypothalamus-pituitary-gonadal axis, secondary sex characteristics and behavior. Several EDCs have been shown to exert a variety of disrupting effects on male reproductive system during critical periods of development, which can have profound permanent effects into adulthood. As our knowledge of mechanistic reproductive endocrinology continues to grow, so also does the list of EDCs, though little is known of the exact pathways and mechanisms of EDC effects.

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APPENDICES

APPENDIX 1

Appendix 1. Correlation matrix between $\log_{10}$ concentrations of metals in livers of 96 adult male river otters collected from Oregon and Washington, 1994-99.

	Ca	Cd	Cu	Fe	K	Mg	Mn	Na	Zn	Hg
Cd	-0.0356	1.00								
	0.7482									
Cu	-0.0076	0.1748	1.00							
	0.9451	0.0886								
Fe	**0.2771**	**0.2955**	0.1383	1.00						
	0.0107	**0.0035**	0.1789							
K	0.0253	-0.1734	0.2005	**-0.2892**	1.00					
	0.8192	0.1147	0.0675	**0.0076**						
Mg	**0.2520**	-0.1623	**0.2610**	-0.1018	**0.8291**	1.00				
	0.0208	0.1401	**0.0165**	0.3569	**<0.0001**					
Mn	0.1752	-0.0513	**0.3761**	-0.1177	**0.5786**	**0.7251**	1.00			
	0.1108	0.6197	**0.0002**	0.2536	**<0.0001**	**<0.0001**				
Na	**0.3278**	0.0457	0.2212	**0.4974**	-0.0882	0.0940	-0.1286	1.00		
	0.0023	0.6796	0.0432	**<0.0001**	0.4249	0.3953	0.2436			
Zn	-0.0130	0.0421	**0.3440**	0.0206	**0.4917**	**0.5618**	**0.6275**	-0.0471	1.00	
	0.9067	0.6841	**0.0006**	0.8420	**<0.0001**	**<0.0001**	**<0.0001**	0.6703		
Hg	**0.2986**	**0.2234**	0.1977	0.0653	0.1699	**0.2627**	**0.4876**	-0.1614	**0.3295**	1.00
	0.0058	**0.0287**	0.0535	0.5272	0.1224	**0.0158**	**<0.0001**	0.1425	**0.0010**	

APPENDIX 2

Appendix 2. Correlation matrix between $\log_{10}$ concentrations of metals and OCs and metabolites, PCBs, PCDDs, and PCDFs in livers of 96 adult male river otters collected from Oregon and Washington, 1994-99.

	QCB	HCB	DDE	DDD	DDT	Mirex	Photmirex	β-HCH	Oxy-chlordane	trans-Nonachlor	cis-Nonachlor
Ca	0.0129	-0.0474	0.0673	0.0703	-0.1104	0.0455	0.1015	-0.0176	0.0478	0.0534	-0.1218
	0.9071	0.6688	0.5429	0.5250	0.3174	0.6814	0.3585	0.8739	0.6662	0.6296	0.2696
Cd	-0.0803	-0.0679	**-0.2181**	-0.1935	-0.0622	-0.0208	0.1876	**0.2713**	**-0.2187**	**-0.2103**	**-0.2232**
	0.4370	0.5112	**0.0328**	0.0590	0.5470	0.8410	0.0672	**0.0075**	**0.0323**	**0.0397**	**0.0288**
Cu	-0.0870	-0.0422	-0.1459	-0.0807	0.1690	-0.1862	0.0644	-0.0799	-0.0879	-0.0623	-0.0023
	0.3991	0.6832	0.1562	0.4347	0.0999	0.0692	0.5332	0.4389	0.3947	0.5467	0.9822
Fe	0.1037	0.0143	0.0536	-0.0308	0.0609	0.0439	**0.2050**	0.1594	0.0492	0.0357	-0.0786
	0.3147	0.8900	0.6043	0.7662	0.5555	0.6710	**0.0451**	0.1207	0.6339	0.7300	0.4463
K	-0.0661	-0.0229	-0.0143	0.0321	-0.0793	**-0.2587**	-0.2057	-0.0847	0.0909	0.0948	0.0352
	0.5503	0.8362	0.8973	0.7720	0.4732	**0.0175**	0.0605	0.4435	0.4111	0.3911	0.7506
Mg	-0.1248	-0.0774	-0.0661	-0.0592	-0.1669	**-0.2182**	-0.1184	0.0077	0.1052	0.0208	-0.0694
	0.2579	0.4843	0.5501	0.5925	0.1293	**0.0461**	0.2835	0.9449	0.3407	0.8513	0.5307
Mn	-0.0437	0.1192	-0.0903	-0.0632	0.0355	-0.0825	0.0198	0.1665	0.1620	0.0879	0.0001
	0.6724	0.2476	0.3817	0.5408	0.7317	0.4245	0.8483	0.1050	0.1148	0.3947	0.9993
Na	0.1239	-0.1076	-0.0080	-0.0644	-0.1541	-0.1202	-0.1351	-0.1267	-0.0615	-0.0476	-0.0863
	0.2616	0.3300	0.9422	0.5605	0.1616	0.2763	0.2203	0.2506	0.5782	0.6674	0.4354
Zn	0.0367	0.0150	**-0.2022**	**-0.2083**	-0.1267	**-0.2664**	0.0021	0.1854	0.0632	-0.0173	-0.0967
	0.7225	0.8845	**0.0482**	**0.0416**	0.2188	**0.0087**	0.9838	0.0705	0.5408	0.8670	0.3488
Hg	-0.0486	0.1892	-0.1191	-0.0579	-0.0183	0.0695	**0.2173**	0.0250	0.1032	0.0817	-0.0466
	0.6383	0.0649	0.2477	0.5750	0.8597	0.5011	**0.0334**	0.8086	0.3169	0.4290	0.6518

Appendix 2. Continued.

	Heptachlor Epoxide	Dieldrin	PCB-49	PCB-52	PCB-60	PCB-66/95	PCB-87	PCB-99	PCB-101	PCB-105	PCB-110
Ca	-0.1501	0.0343	-0.1136	-0.0393	-0.0502	-0.1720	-0.0238	-0.0922	-0.1667	-0.1689	**-0.2590**
	0.1729	0.7571	0.3035	0.7224	0.6504	0.1177	0.8300	0.4040	0.1296	0.1246	**0.0174**
Cd	-0.1854	**-0.2447**	**-0.2954**	-0.1928	-0.0055	**-0.2428**	-0.0084	**-0.2004**	**-0.2511**	0.0359	-0.1647
	0.0706	**0.0163**	**0.0035**	0.0599	0.9577	**0.0171**	0.9355	**0.0503**	**0.0136**	0.7282	0.1088
Cu	-0.0450	-0.1023	-0.0914	0.0057	-0.1023	0.0657	0.0186	-0.1352	-0.0227	0.1410	0.0826
	0.6631	0.3213	0.3758	0.9568	0.3212	0.5247	0.8572	0.1890	0.8266	0.1706	0.4237
Fe	-0.0139	0.0508	-0.1075	-0.0798	0.0004	-0.1348	0.0004	-0.1803	**-0.2041**	-0.0569	-0.1330
	0.8931	0.6232	0.2973	0.4397	0.9973	0.1903	0.9968	0.0787	**0.0461**	0.5820	0.1963
K	0.1888	0.0954	0.0548	0.0705	-0.1477	0.0063	-0.0326	0.0994	0.1453	-0.0938	-0.0836
	0.0854	0.3881	0.6207	0.5237	0.1801	0.9544	0.7682	0.3683	0.1872	0.3961	0.4494
Mg	0.0675	-0.0101	-0.0204	-0.0070	-0.1114	-0.1498	-0.0723	0.0345	0.0382	-0.1411	-0.1671
	0.5419	0.9276	0.8536	0.9498	0.3129	0.1737	0.5136	0.7552	0.7299	0.2005	0.1287
Mn	0.0306	-0.0593	-0.0352	0.0810	0.0764	-0.1222	-0.0649	0.0381	0.0264	-0.1883	-0.1394
	0.7676	0.5659	0.7337	0.4330	0.4596	0.2354	0.5300	0.7126	0.7985	0.0662	0.1755
Na	-0.0118	0.1051	-0.1517	-0.0022	-0.2068	-0.0705	0.0985	-0.1022	-0.0484	0.1231	-0.0539
	0.9155	0.3414	0.1683	0.9845	0.0592	0.5239	0.3727	0.3552	0.6619	0.2647	0.6262
Zn	0.0493	-0.1340	-0.1519	-0.0472	-0.0278	-0.1398	-0.0327	-0.0312	-0.0899	-0.1350	-0.1402
	0.6337	0.1931	0.1396	0.6481	0.7877	0.1742	0.7515	0.7631	0.3837	0.1896	0.1730
Hg	-0.0779	-0.1191	-0.1513	-0.0867	0.0666	-0.1741	-0.1977	-0.0530	-0.1073	-0.1691	-0.1572
	0.4506	0.2477	0.1412	0.4009	0.5189	0.0898	0.0535	0.6078	0.2981	0.0995	0.1262

Appendix 2. Continued.

	PCB-118	PCB-129	PCB-138	PCB-146	PCB-149	PCB-151	PCB-153	PCB-170/190	PCB-171	PCB-172	PCB-180
Ca	-0.1715	-0.1331	-0.1481	-0.1016	-0.0622	-0.1770	-0.1072	-0.1881	-0.1450	-0.0765	-0.1425
	0.1189	0.2276	0.1789	0.3577	0.5742	0.1074	0.3319	0.0866	0.1882	0.4892	0.1961
Cd	-0.1384	-0.1953	-0.1920	**-0.2507**	-0.1777	**-0.2917**	**-0.2051**	**-0.2233**	**-0.2419**	**-0.2388**	**-0.2506**
	0.1787	0.0552	0.0609	**0.0137**	0.0833	**0.0039**	**0.0450**	**0.0288**	**0.0176**	**0.0191**	**0.0138**
Cu	0.1630	-0.1459	-0.1518	-0.1176	0.0134	**-0.1583**	-0.1824	-0.1785	-0.1240	-0.1932	-0.1964
	0.1125	0.1561	0.1399	0.2537	0.8973	**0.1234**	0.0752	0.0818	0.2288	0.0593	0.0551
Fe	-0.0837	**-0.2426**	-0.1014	-0.1847	-0.0634	-0.2735	-0.1044	-0.1788	-0.1622	-0.1700	-0.1452
	0.4176	**0.0173**	0.3257	0.0716	0.5397	0.0070	0.3113	0.0814	0.1143	0.0977	0.1580
K	0.0189	0.0180	0.0805	0.1246	-0.1292	-0.0891	0.0762	0.1061	0.1188	0.0215	0.1022
	0.8642	0.8709	0.4668	0.2589	0.2415	0.4201	0.4908	0.3369	0.2819	0.8462	0.3551
Mg	-0.0730	0.0189	0.0428	0.1020	-0.1982	-0.1626	0.0591	0.0899	0.0545	0.0178	0.0797
	0.5091	0.8642	0.6990	0.3559	0.0707	0.1395	0.5936	0.4163	0.6225	0.8722	0.4709
Mn	-0.0059	0.0424	-0.0103	0.0590	-0.0914	-0.1622	0.0560	-0.0260	0.0254	-0.0345	-0.0137
	0.9544	0.6814	0.9207	0.5683	0.3761	0.1143	0.5876	0.8012	0.8063	0.7386	0.8943
Na	0.0308	-0.1032	0.0038	-0.0242	-0.0310	-0.1082	-0.0147	0.0469	0.0198	0.0417	0.0262
	0.7810	0.3501	0.9728	0.8267	0.7795	0.3270	0.8948	0.6721	0.8582	0.7062	0.8128
Zn	-0.0507	0.0126	0.0301	0.0013	-0.2277	**-0.2026**	0.0554	0.0233	-0.0099	-0.0645	0.0050
	0.6237	0.9031	0.7712	0.9897	0.0257	**0.0478**	0.5919	0.8217	0.9237	0.5328	0.9617
Hg	-0.1144	-0.1681	-0.1880	-0.1008	-0.1245	**-0.2575**	-0.1121	**-0.2622**	-0.1054	-0.1802	**-0.2173**
	0.2672	0.1017	0.0667	0.3286	0.2267	**0.0113**	0.2770	**0.0099**	0.3068	0.0790	**0.0335**

Appendix 2. Continued.

	PCB-182/187	PCB-183	PCB-194	PCB-195	PCB-200	PCB-201	PCB-203	PCB-206	∑PCB	PCB-77	PCB-81
Ca	-0.0682	-0.0104	-0.1683	-0.1128	-0.1510	-0.1447	-0.1185	-0.2024	-0.1439	0.1077	0.0022
	0.5378	0.3467	0.1260	0.3071	0.1705	0.1890	0.2831	0.0649	0.1917	0.3295	0.9843
Cd	**-0.3117**	**-0.2660**	**-0.2299**	**-0.2156**	-0.1633	-0.2913	**-0.2779**	**-0.2143**	**-0.2407**	-0.0839	0.1219
	0.0020	**0.0088**	**0.0242**	**0.0349**	0.1120	0.0040	**0.0061**	**0.0360**	**0.0181**	0.4166	0.2367
Cu	-0.1849	-0.1810	**-0.2115**	-0.1160	-0.0855	-0.1958	-0.1890	**-0.2126**	-0.1899	0.1195	0.1096
	0.0714	0.0776	**0.0385**	0.2604	0.4074	0.0559	0.0652	**0.0375**	0.0638	0.2463	0.2877
Fe	-0.0784	-0.0836	**-0.2019**	-0.1463	-0.1616	-0.1977	-0.0991	**-0.2019**	-0.1379	0.1145	-0.0177
	0.4478	0.4180	**0.0485**	0.1549	0.1158	0.0535	0.3367	**0.0485**	0.1805	0.2668	0.8640
K	0.0738	0.0690	0.1318	0.1162	0.0557	0.1205	0.0996	0.1289	0.0867	-0.0973	-0.1172
	0.5046	0.5328	0.2322	0.2926	0.6147	0.2749	0.3674	0.2425	0.4330	0.3784	0.2883
Mg	0.0510	0.0531	0.1028	0.0732	0.0277	0.1179	0.0621	0.0891	0.0613	-0.1314	-0.1636
	0.6450	0.6314	0.3523	0.5079	0.8025	0.2856	0.5745	0.4201	0.5796	0.2335	0.1371
Mn	-0.0780	-0.0080	0.0001	-0.0187	-0.0125	-0.0619	-0.0411	-0.0162	-0.0037	-0.1055	-0.0735
	0.4501	0.9385	0.9990	0.8565	0.9036	0.5491	0.6906	0.8756	0.9715	0.3064	0.4769
Na	0.1058	0.0483	0.0244	0.0496	0.0619	0.0827	0.0664	0.0204	0.0122	0.0900	-0.0957
	0.3381	0.6624	0.8257	0.6540	0.5757	0.4547	0.5482	0.8539	0.9125	0.4154	0.3866
Zn	-0.0386	0.0101	0.0443	-0.0504	-0.0401	-0.0105	-0.0222	0.0816	0.0191	-0.0784	**-0.2186**
	0.7090	0.9225	0.6683	0.6259	0.6980	0.9192	0.8298	0.4292	0.8536	0.4480	**0.0323**
Hg	**-0.2143**	-0.1910	**-0.2148**	**-0.2112**	-0.1501	**-0.2779**	**-0.2164**	**-0.2084**	-0.1916	-0.1446	0.0893
	0.0360	0.0623	**0.0355**	**0.0389**	0.1445	**0.0061**	**0.0342**	**0.0416**	0.0614	0.1597	0.3867

Appendix 2. Continued.

	PCB-126	PCB-169	total TCDD	1,6,7,8-H6CDD	total H6CDD	1,2,3,4,6,7,8-H7CDD	total H7CDD	OCDD	total TCDF	1,2,4,7,8-PCDF	total PCDF
Ca	-0.1340	-0.0520	0.0081	-0.1002	-0.0168	0.0453	-0.0203	-0.1289	0.0119	-0.0048	0.0221
	0.2243	0.6385	0.9417	0.3646	0.8792	0.6826	0.8545	0.2425	0.9146	0.9655	0.8416
Cd	-0.1601	-0.1147	-0.0850	**-0.2956**	**-0.2508**	**-0.2499**	**-0.2398**	**-0.2586**	-0.1567	-0.0675	0.0626
	0.1193	0.2656	0.4103	**0.0035**	**0.0137**	**0.0141**	**0.0186**	**0.0192**	0.1273	0.5133	0.5447
Cu	0.1232	0.0131	-0.1355	0.0221	-0.0362	-0.1668	-0.1624	-0.1058	0.0295	-0.1466	-0.0934
	0.2316	0.8994	0.1881	0.8309	0.7263	0.1044	0.1138	0.3048	0.7754	0.1541	0.3655
Fe	-0.1015	-0.0366	-0.0593	**-0.2595**	**-0.2040**	-0.1541	-0.1859	-0.1924	-0.0499	-0.1002	-0.0990
	0.3250	0.7232	0.5660	**0.0107**	**0.0463**	0.1338	0.0697	0.0604	0.6294	0.3314	0.3372
K	0.0384	0.0634	0.0834	0.2041	0.1355	0.1580	0.0826	0.0422	-0.0629	-0.0827	-0.0512
	0.7289	0.5670	0.4508	0.0626	0.2191	0.1513	0.4553	0.7030	0.5699	0.4543	0.6436
Mg	-0.0064	0.0954	-0.0554	0.1100	0.0159	0.1097	0.0273	-0.0215	-0.1133	-0.0539	-0.0273
	0.9538	0.3880	0.6170	0.3192	0.8856	0.3208	0.8055	0.8463	0.3047	0.6260	0.8050
Mn	-0.0096	0.1440	-0.1003	-0.0816	-0.1797	-0.1080	-0.1668	-0.1197	-0.1548	-0.1124	-0.1536
	0.9264	0.1615	0.3308	0.4295	0.0798	0.2947	0.1043	0.2452	0.1320	0.2754	0.1351
Na	0.1208	-0.0418	0.0932	0.0908	0.1440	0.0530	-0.0521	0.0345	-0.0728	-0.0752	0.0100
	0.2735	0.7056	0.3990	0.4114	0.1913	0.6319	0.6380	0.7556	0.5102	0.4967	0.9279
Zn	-0.0007	0.0671	-0.0100	-0.0505	-0.1686	-0.1515	-0.1668	-0.0563	-0.1573	**-0.2773**	**-0.2138**
	0.9947	0.5160	0.9230	0.6249	0.1006	0.1405	0.1043	0.5859	0.1260	**0.0062**	**0.0365**
Hg	**-0.3047**	-0.0275	-0.0304	**-0.2151**	**-0.2905**	-0.2549	**-0.2812**	**-0.3073**	-0.0650	-0.1737	**-0.2426**
	0.0025	0.7904	0.7685	**0.0353**	**0.0041**	0.0122	**0.0055**	**0.0023**	0.5294	0.0906	**0.0173**

Appendix 2. Continued.

	1,2,3,4,7,8-H6CDF	total H6CDF	1,2,3,4,6,7,8-H7CDF	total H7CDF	OCDF
Ca	0.0818	0.0404	-0.1079	-0.1330	-0.1052
	0.4596	0.7151	0.3284	0.2277	0.3410
Cd	-0.1918	-0.1269	-0.1901	**-0.2040**	-0.1592
	0.0613	0.2180	0.0636	**0.0462**	0.1214
Cu	-0.1333	-0.0176	-0.0568	-0.0228	-0.0399
	0.1955	0.8649	0.5823	0.8255	0.6993
Fe	-0.1631	-0.1306	-0.1837	**-0.2345**	**-0.2855**
	0.1123	0.2049	0.0732	**0.0215**	**0.0048**
K	0.0969	0.1054	0.2006	0.1582	-0.0181
	0.3805	0.3399	0.0673	0.1506	0.8705
Mg	0.0496	0.0700	0.0939	0.0636	-0.0773
	0.6542	0.5267	0.3957	0.5658	0.4848
Mn	-0.1267	-0.1029	-0.1318	-0.1030	-0.1498
	0.2186	0.3186	0.2006	0.3178	0.1452
Na	-0.0963	0.0698	0.1906	0.1640	0.0883
	0.3837	0.5279	0.0825	0.1361	0.4242
Zn	**-0.2964**	-0.1892	-0.1064	-0.0694	-0.1023
	0.0034	0.0649	0.3024	0.5018	0.3212
Hg	-0.1703	-0.1988	**-0.3139**	**-0.3200**	**-0.2255**
	0.0972	0.0522	**0.0018**	**0.0015**	**0.0272**

APPENDIX 3

Appendix 3. Correlation matrix between $\log_{10}$ liver concentrations of OCs and metabolites, PCBs, PCDDs, and PCDFs of 96 adult river otter males collected from Oregon and Washington, 1994-99.

Contaminant	QCB	HCB	DDE	DDD	DDT	Mirex	Photmirex	β-HCH
QCB	1.00							
HCB	0.47378	1.00						
	<0.0001							
DDE	0.41547	0.56287	1.00					
	<0.0001	<0.0001						
DDD	0.39755	0.50516	0.84098	1.00				
	<0.0001	<0.0001	<0.0001					
DDT	0.23187	0.50420	0.43330	0.38406	1.00			
	0.0230	<0.0001	<0.0001	0.0001				
Mirex	0.10275	0.44087	0.48146	0.50413	0.46300	1.00		
	0.3192	<0.0001	<0.0001	<0.0001	<0.0001			
Photomirex	-0.13282	0.21524	-0.0127	-0.0481	0.26805	0.38241	1.00	
	0.1970	0.0352	0.9024	0.6417	<0.0001	0.0001		
β-HCH	0.11488	0.12240	0.01899	0.06303	0.06099	0.19126	0.16747	1.00
	0.2651	0.2348	0.8543	0.5418	0.5550	0.0620	0.1029	
Oxychlordane	0.30896	0.50139	0.71552	0.64924	0.38751	0.39756	-0.0273	0.20760
	0.0022	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.7915	0.0424
trans-Nonachlor	0.40070	0.61292	0.80466	0.80099	0.42658	0.52342	0.09073	0.18955
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3793	0.0644
cis-Nonochlor	0.37564	0.53542	0.76369	0.80972	0.46729	0.46426	-0.00021	0.12493
	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.9839	0.2252
Heptachlor Epoxide	0.46645	0.46397	0.70892	0.70949	0.27679	0.21089	-0.2216	0.21672
	<0.0001	<0.0001	<0.0001	<0.0001	0.0063	0.0392	0.0300	0.0339
Dieldrin	0.51619	0.45395	0.77166	0.81839	0.21004	0.24435	-0.2919	0.08702
	<0.0001	<0.0001	<0.0001	<0.0001	0.0400	0.0164	0.0039	0.3992

Appendix 3. Continued.

Contaminant	Oxy-chlordane	trans-Nonachlor	cis-Nonachlor	Heptachlor Epoxide	Dieldrin	PCB-49	PCB-52	PCB-60
QCB								
HCB								
DDE								
DDD								
DDT								
Mirex								
Photomirex								
β-HCH								
Oxychlordane	1.00							
trans-Nonachlor	0.85167 <0.0001	1.00						
cis-Nonochlor	0.72183 <0.0001	0.85833 <0.0001	1.00					
Heptachlor Epoxide	0.75351 <0.0001	0.78483 <0.0001	0.79686 <0.0001	1.00				
Dieldrin	0.72319 <0.0001	0.79179 <0.0001	0.78166 <0.0001	0.85900 <0.0001	1.00			

Appendix 3. Continued.

Contaminant	QCB	HCB	DDE	DDD	DDT	Mirex	Photmirex	β-HCH
PCB-49	0.28031	0.37028	0.45791	0.45780	0.52897	0.49565	0.01537	0.00147
	0.0057	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	0.8819	0.9887
PCB-52	0.31461	0.59789	0.57378	0.63317	0.44026	0.56474	0.10237	0.20192
	0.0018	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3210	0.0485
PCB-60	0.16086	0.49859	0.42932	0.45803	0.34932	0.70841	0.29577	0.31431
	0.1174	<0.0001	<0.0001	<0.0001	0.0005	<0.0001	0.0034	0.0018
PCB-66/95	0.32884	0.47800	0.62511	0.62484	0.55177	0.41899	-0.0008	-0.04436
	0.0011	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.9942	0.6678
PCB-87	0.11169	0.28808	0.17642	0.14923	0.31360	0.47201	0.15016	0.17759
	0.2786	0.0044	0.0855	0.1468	0.0019	<0.0001	0.1442	0.0835
PCB-99	0.25387	0.46293	0.61572	0.57003	0.39213	0.47675	-0.09430	0.28112
	0.0126	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3608	0.0055
PCB-101	0.31233	0.48212	0.65986	0.68148	0.40751	0.46794	-0.13397	0.11925
	0.0019	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1932	0.2472
PCB-105	0.13050	0.13445	0.14891	0.20961	0.15110	0.30599	-0.04488	0.04245
	0.2051	0.1915	0.1476	0.0404	0.1417	0.0024	0.6642	0.6813
PCB-110	0.06607	0.34267	0.29473	0.32045	0.49646	0.43575	0.08926	0.03415
	0.5224	0.0006	0.0036	0.0015	<0.0001	<0.0001	0.3871	0.7411
PCB-118	0.34615	0.39707	0.41324	0.46137	0.37557	0.34294	-0.10434	0.12462
	0.0006	<0.0001	<0.0001	<0.0001	0.0002	0.0006	0.3117	0.2264
PCB-129	0.28673	0.30618	0.36097	0.35566	0.31157	0.47928	-0.07296	0.29865
	0.0046	0.0024	0.0003	0.0004	0.0020	<0.0001	0.4799	0.0031
PCB-138	0.28775	0.37101	0.65671	0.55145	0.34108	0.38319	-0.26885	0.24462
	0.0045	0.0002	<0.0001	<0.0001	0.0007	0.0001	0.0081	0.0163
PCB-146	0.33128	0.41546	0.64273	0.58082	0.35829	0.45554	-0.19799	0.26035
	0.0010	<0.0001	<0.0001	<0.0001	0.0003	<0.0001	0.0532	0.0104

Appendix 3. Continued.

Contaminant	Oxy-chlordane	trans-Nonachlor	cis-Nonachlor	Heptachlor Epoxide	Dieldrin	PCB-49	PCB-52	PCB-60
PCB-49	0.33707	0.41690	0.44456	0.23937	0.29868	1.00		
	0.0008	<0.0001	<0.0001	0.0188	0.0031			
PCB-52	0.47534	0.66509	0.71492	0.48038	0.48318	0.57393	1.00	
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		
PCB-60	0.38497	0.50461	0.47718	0.24905	0.19947	0.50401	0.66234	1.00
	0.0001	<0.0001	<0.0001	0.0144	0.0514	<0.0001	<0.0001	
PCB-66/95	0.53972	0.65765	0.75941	0.59762	0.54914	0.54514	0.70720	0.42216
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-87	0.18629	0.27207	0.29074	0.16728	0.09549	0.35408	0.53325	0.52889
	0.0692	0.0073	0.0041	0.1033	0.3547	0.0004	<0.0001	<0.0001
PCB-99	0.68023	0.75781	0.65911	0.57877	0.53606	0.45450	0.62532	0.55177
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-101	0.59763	0.72762	0.77484	0.63692	0.62631	0.53089	0.74215	0.56054
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-105	0.02074	0.14930	0.22305	0.15367	0.14290	0.25352	0.38399	0.29411
	0.8411	0.1465	0.0289	0.1350	0.1649	0.0127	0.0001	0.0036
PCB-110	0.30220	0.36958	0.48556	0.20278	0.16794	0.47520	0.57310	0.52073
	0.0028	<0.0001	<0.0001	0.0475	0.1019	<0.0001	<0.0001	<0.0001
PCB-118	0.37429	0.48777	0.53071	0.36188	0.35762	0.50351	0.68112	0.45980
	0.0002	<0.0001	<0.0001	0.0003	0.0003	<0.0001	<0.0001	<0.0001
PCB-129	0.34540	0.42855	0.45610	0.32499	0.29363	0.50427	0.69996	0.58971
	0.0006	<0.0001	<0.0001	0.0012	0.0037	<0.0001	<0.0001	<0.0001
PCB-138	0.71930	0.69611	0.67479	0.66364	0.62034	0.43973	0.56755	0.46119
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-146	0.66939	0.73621	0.67374	0.61993	0.59041	0.50149	0.65303	0.53556
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Appendix 3. Continued.

Contaminant	PCB-66/95	PCB-87	PCB-99	PCB-101	PCB-105	PCB-110	PCB-118	PCB-129
PCB-49								
PCB-52								
PCB-60								
PCB-66/95	1.00							
PCB-87	0.46540 <0.0001	1.00						
PCB-99	0.61282 <0.0001	0.45632 <0.0001	1.00					
PCB-101	0.73624 <0.0001	0.48649 <0.0001	0.78869 <0.0001	1.00				
PCB-105	0.27582 0.0065	0.47447 <0.0001	0.26015 0.0105	0.32868 0.0011	1.00			
PCB-110	0.64553 <0.0001	0.56761 <0.0001	0.57113 <0.0001	0.58760 <0.0001	0.41935 <0.0001	1.00		
PCB-118	0.59144 <0.0001	0.48920 <0.0001	0.60300 <0.0001	0.71132 <0.0001	0.52904 <0.0001	0.66558 <0.0001	1.00	
PCB-129	0.47855 <0.0001	0.53125 <0.0001	0.67131 <0.0001	0.69084 <0.0001	0.31305 0.0019	0.46330 <0.0001	0.56510 <0.0001	1.00
PCB-138	0.61824 <0.0001	0.42317 <0.0001	0.87542 <0.0001	0.76239 <0.0001	0.23777 0.0197	0.52041 <0.0001	0.51222 <0.0001	0.67604 <0.0001
PCB-146	0.62236 <0.0001	0.46842 <0.0001	0.94253 <0.0001	0.84124 <0.0001	0.26767 0.0084	0.51688 <0.0001	0.60122 <0.0001	0.77998 <0.0001

Appendix 3. Continued.

Contaminant	PCB-138	PCB-146	PCB-149	PCB-151	PCB-153	PCB-170/190	PCB-171	PCB-172
PCB-49								
PCB-52								
PCB-60								
PCB-66/95								
PCB-87								
PCB-99								
PCB-101								
PCB-105								
PCB-110								
PCB-118								
PCB-129								
PCB-138	1.00							
PCB-146	0.91446 <0.0001	1.00						

Appendix 3. Continued.

Contaminant	QCB	HCB	DDE	DDD	DDT	Mirex	Photmirex	β-HCH
PCB-149	0.20215	0.35704	0.48724	0.52702	0.54331	0.63257	0.16657	0.06048
	0.0482	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	0.1048	0.5583
PCB-151	0.19461	0.29898	0.37666	0.42480	0.44332	0.48468	-0.05647	0.06048
	0.0574	0.0031	0.0002	<0.0001	<0.0001	<0.0001	0.5847	0.0083
PCB-153	0.29206	0.40625	0.63197	0.52140	0.37212	0.41456	-0.22575	0.30656
	0.0039	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.0270	0.0024
PCB-170/190	0.21757	0.16598	0.48934	0.36491	0.18421	0.19010	-0.46662	0.19499
	0.0332	0.1060	<0.0001	0.0003	0.0724	0.0636	<0.0001	0.0569
PCB-171	0.28914	0.34788	0.60305	0.52599	0.34008	0.40109	-0.22406	0.15455
	0.0043	0.0005	<0.0001	<0.0001	0.0007	<0.0001	0.0282	0.1327
PCB-172	0.36141	0.35612	0.59965	0.53722	0.25327	0.42873	-0.24026	0.23412
	0.0003	0.0004	<0.0001	<0.0001	0.0128	<0.0001	0.0184	0.0217
PCB-180	0.27283	0.26929	0.57864	0.43767	0.26097	0.28173	-0.38629	0.18951
	0.0072	0.0080	<0.0001	<0.0001	0.0102	0.0054	0.0001	0.0644
PCB-182/187	0.34850	0.35085	0.67493	0.58587	0.30887	0.39455	-0.32883	0.13793
	0.0005	0.0005	<0.0001	<0.0001	0.0022	<0.0001	0.0011	0.1802
PCB-183	0.30368	0.37746	0.66321	0.54907	0.35579	0.40309	-0.27405	0.23809
	0.0026	0.0001	<0.0001	<0.0001	0.0004	<0.0001	0.0069	0.0195
PCB-194	0.26251	0.19432	0.47908	0.34697	0.15478	0.19040	-0.46127	0.18785
	0.0098	0.0578	<0.0001	0.0005	0.1321	0.0631	<0.0001	0.0668
PCB-195	0.29228	0.23736	0.55421	0.46212	0.24711	0.26820	-0.36283	0.16873
	0.0039	0.0199	<0.0001	<0.0001	0.0152	0.0082	0.0003	0.1003
PCB-200	0.21925	0.27265	0.51424	0.46377	0.25773	0.35700	-0.28441	0.21321
	0.0319	0.0072	<0.0001	<0.0001	0.0112	0.0004	0.0050	0.0370
PCB-201	0.23994	0.15592	0.50400	0.41063	0.17845	0.23249	-0.46709	0.13803
	0.0185	0.1293	<0.0001	<0.0001	0.0819	0.0226	<0.0001	0.1799

Appendix 3. Continued.

Contaminant	Oxy-chlordane	trans-Nonachlor	cis-Nonachlor	Heptachlor Epoxide	Dieldrin	PCB-49	PCB-52	PCB-60
PCB-149	0.42884	0.55549	0.65253	0.37389	0.37893	0.54847	0.66147	0.53727
	<0.0001	<0.0001	<0.0001	0.0002	0.0001	<0.0001	<0.0001	<0.0001
PCB-151	0.29828	0.39175	0.50674	0.30474	0.29866	0.60700	0.58855	0.50588
	0.0032	<0.0001	<0.0001	0.0025	0.0031	<0.0001	<0.0001	<0.0001
PCB-153	0.71561	0.67840	0.63239	0.61403	0.57041	0.46700	0.57054	0.49466
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-170/190	0.50309	0.43609	0.43619	0.52204	0.49000	0.34887	0.36511	0.25775
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0005	0.0003	0.0112
PCB-171	0.62842	0.68716	0.64105	0.59809	0.58939	0.47468	0.56503	0.45679
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-172	0.51408	0.55474	0.52569	0.52406	0.53025	0.48790	0.60710	0.50803
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-180	0.58788	0.53715	0.51211	0.55976	0.53671	0.42141	0.43484	0.33257
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0009
PCB-182/187	0.63928	0.66363	0.70071	0.67529	0.67736	0.49644	0.62245	0.40115
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-183	0.70946	0.68168	0.67706	0.65774	0.63274	0.48690	0.59275	0.44852
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-194	0.47785	0.42525	0.40356	0.49759	0.47273	0.37670	0.35585	0.25848
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0004	0.0110
PCB-195	0.57709	0.56372	0.50840	0.56402	0.57025	0.42537	0.42954	0.31712
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0016
PCB-200	0.57319	0.58192	0.52368	0.53924	0.50748	0.39492	0.49369	0.43732
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-201	0.49745	0.45321	0.49264	0.53002	0.51525	0.42282	0.45158	0.30691
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0024

Appendix 3. Continued.

Contaminant	PCB-66/95	PCB-87	PCB-99	PCB-101	PCB-105	PCB-110	PCB-118	PCB-129
PCB-149	0.76866	0.60761	0.56727	0.66908	0.39002	0.71213	0.61035	0.48132
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-151	0.64542	0.59879	0.51701	0.60879	0.33653	0.60626	0.54258	0.57520
	<0.0001	<0.0001	<0.0001	<0.0001	0.0008	<0.0001	<0.0001	<0.0001
PCB-153	0.58447	0.42856	0.89148	0.74320	0.15969	0.50947	0.51878	0.71508
	<0.0001	<0.0001	<0.0001	<0.0001	0.1202	<0.0001	<0.0001	<0.0001
PCB-170/190	0.44103	0.34171	0.74436	0.62403	0.15580	0.35966	0.40272	0.66716
	<0.0001	0.0007	<0.0001	<0.0001	0.1296	0.0003	<0.0001	<0.0001
PCB-171	0.59887	0.40733	0.84854	0.82305	0.21359	0.48382	0.53976	0.73139
	<0.0001	<0.0001	<0.0001	<0.0001	0.0367	<0.0001	<0.0001	<0.0001
PCB-172	0.50440	0.42235	0.75845	0.75709	0.27168	0.40949	0.51425	0.84340
	<0.0001	<0.0001	<0.0001	<0.0001	0.0074	<0.0001	<0.0001	<0.0001
PCB-180	0.51381	0.35585	0.80341	0.66813	0.12990	0.39985	0.43417	0.67952
	<0.0001	0.0004	<0.0001	<0.0001	0.2072	<0.0001	<0.0001	<0.0001
PCB-182/187	0.64262	0.41369	0.76560	0.78547	0.22426	0.45902	0.52166	0.71010
	<0.0001	<0.0001	<0.0001	<0.0001	0.0280	<0.0001	<0.0001	<0.0001
PCB-183	0.62208	0.42932	0.84818	0.76206	0.17634	0.49912	0.51029	0.71832
	<0.0001	<0.0001	<0.0001	<0.0001	0.0857	<0.0001	<0.0001	<0.0001
PCB-194	0.41803	0.30282	0.73640	0.60628	0.12328	0.31727	0.39343	0.67585
	<0.0001	0.0027	<0.0001	<0.0001	0.2315	0.0016	<0.0001	<0.0001
PCB-195	0.49266	0.33368	0.81194	0.68167	0.19974	0.39828	0.44540	0.68937
	<0.0001	0.0009	<0.0001	<0.0001	0.0510	<0.0001	<0.0001	<0.0001
PCB-200	0.53154	0.45839	0.88100	0.72304	0.36426	0.51611	0.55847	0.69772
	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	<0.0001
PCB-201	0.49131	0.38000	0.74702	0.69449	0.22627	0.40899	0.45161	0.72885
	<0.0001	<0.0001	<0.0001	<0.0001	0.0266	<0.0001	<0.0001	<0.0001

Appendix 3. Continued.

Contaminant	PCB-138	PCB-146	PCB-149	PCB-151	PCB-153	PCB-170/190	PCB-171	PCB-172
PCB-149	0.54194	0.55632	1.00					
	<0.0001	<0.0001						
PCB-151	0.51651	0.55895	0.71103	1.00				
	<0.0001	<0.0001	<0.0001					
PCB-153	0.96156	0.91838	0.52575	0.52231	1.00			
	<0.0001	<0.0001	<0.0001	<0.0001				
PCB-170/190	0.89385	0.82765	0.34602	0.44484	0.90325	1.00		
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001			
PCB-171	0.87539	0.91490	0.53759	0.54129	0.88202	0.82178	1.00	
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		
PCB-172	0.80973	0.88078	0.43763	0.52838	0.82900	0.81306	0.82939	1.00
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
PCB-180	0.92136	0.86507	0.40358	0.47893	0.94629	0.98039	0.85469	0.83249
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-182/187	0.89371	0.88519	0.55611	0.57181	0.86765	0.83799	0.87971	0.83607
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-183	0.96282	0.91793	0.53938	0.55540	97293	0.91142	0.90030	0.85032
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-194	0.85868	0.81785	0.30815	0.42307	0.89023	0.98052	0.82208	0.82270
	<0.0001	<0.0001	0.0023	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-195	0.88892	0.88229	0.40283	0.42114	0.88706	0.91205	0.88868	0.85061
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-200	0.87683	0.89141	0.48742	0.50298	0.87355	0.85028	0.89723	0.82492
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-201	0.87953	0.87155	0.41253	0.51660	0.86761	0.93553	0.85525	0.86083
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Appendix 3. Continued.

Contaminant	PCB-180	PCB-182/187	PCB-183	PCB-194	PCB-195	PCB-200	PCB-201	PCB-203
PCB-149								
PCB-151								
PCB-153								
PCB-170/190								
PCB-171								
PCB-172								
PCB-180	1.00							
PCB-182/187	0.85688 <0.0001	1.00						
PCB-183	0.94602 <0.0001	0.94476 <0.0001	1.00					
PCB-194	0.97678 <0.0001	0.81543 <0.0001	0.89119 <0.0001	1.00				
PCB-195	0.91794 <0.0001	0.83408 <0.0001	0.89827 <0.0001	0.90795 <0.0001	1.00			
PCB-200	0.85898 <0.0001	0.79223 <0.0001	0.85576 <0.0001	0.83838 <0.0001	0.90310 <0.0001	1.00		
PCB-201	0.91598 <0.0001	0.92323 <0.0001	0.91418 <0.0001	0.91835 <0.0001	0.89447 <0.0001	0.84504 <0.0001	1.00	

Appendix 3. Continued.

Contaminant	QCB	HCB	DDE	DDD	DDT	Mirex	Photmirex	β-HCH
PCB-203	0.32491	0.32448	0.64727	0.54526	0.30022	0.33272	-0.37224	0.16944
	0.0012	0.0013	<0.0001	<0.0001	0.0030	0.0009	0.0002	0.0989
PCB-206	0.23000	0.17859	0.44755	0.36634	0.14162	0.17720	-0.49979	0.20260
	0.0242	0.0817	<0.0001	0.0002	0.1687	0.0841	<0.0001	0.0477
SPCB	0.29227	0.34826	0.62722	0.51400	0.32595	0.36321	-0.31827	0.23589
	0.0039	0.0005	<0.0001	<0.0001	0.0012	0.0003	0.0016	0.0270
PCB–77	0.00507	0.05825	0.00761	-0.01775	0.13209	-0.07193	0.21131	0.14802
	0.9609	0.5730	0.9414	0.8638	0.1995	0.4862	0.0388	0.1501
PCB-81	0.05035	0.22923	0.16180	0.20850	0.30797	0.22052	0.13397	0.06233
	0.6262	0.0247	0.1153	0.0415	0.0023	0.0308	0.1931	0.5463
PCB-126	0.03044	0.10787	0.16183	0.09427	0.25209	0.11027	0.02573	0.00907
	0.7684	0.2955	0.1152	0.3609	0.0132	0.2848	0.8035	0.9301
PCB-169	0.08044	0.26844	0.29161	0.21634	0.20617	0.21376	-0.01141	0.29244
	0.4360	0.0082	0.0039	0.0343	0.0439	0.0365	0.9121	0.0038
total TCDD	-0.04832	0.00975	0.02274	-0.05357	-0.12552	-0.03685	-0.13251	-0.01017
	0.6402	0.9249	0.8260	0.6042	0.2230	0.7215	0.1981	0.9216
1,2,3,6,7,8-H6CDD	-0.06999	-0.00421	0.04352	-0.01785	0.00527	-0.12924	-0.31281	-0.05736
	0.4980	0.9675	0.6737	0.8630	0.9594	0.2095	0.0019	0.5788
total H6CDD	0.02049	-0.08652	0.06614	0.05031	0.01448	-0.09413	-0.28501	-0.00968
	0.8429	0.4019	0.5220	0.6264	0.8887	0.3616	0.0049	0.9254
1,2,3,4,6,7,8-H7CDD	0.06616	-0.03109	0.16634	0.22819	-0.13159	-0.11656	-0.50178	0.03876
	0.5219	0.7636	0.1053	0.0253	0.2013	0.2581	<0.0001	0.7077
total H7CDD	0.09973	-0.00953	0.20260	0.25028	-0.10287	-0.08542	-0.49745	0.05880
	0.3336	0.9265	0.0477	0.0139	0.3186	0.4079	<0.0001	0.5693
OCDD	0.19877	0.02732	0.22022	0.29077	-0.06390	-0.05226	-0.40928	0.21534
	0.0522	0.7916	0.0311	0.0041	0.5362	0.6131	<0.0001	0.0351

Appendix 3. Continued.

Contaminant	Oxy-chlordane	trans-Nonachlor	cis-Nonachlor	Heptachlor Epoxide	Dieldrin	PCB-49	PCB-52	PCB-60
PCB-203	0.67296	0.64781	0.64337	0.66714	0.66762	0.47736	0.52488	-0.07744
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.4533
PCB-206	0.48455	0.41231	0.43995	0.51978	0.48796	0.37452	0.36673	0.21608
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0002	0.0345
SPCB	0.66306	0.63410	0.61729	0.62430	0.59127	0.45918	0.53793	0.18821
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0663
PCB–77	-0.05764	0.02290	0.06590	0.03001	0.01781	-0.05266	0.08810	0.22797
	0.5770	0.8248	0.5235	0.7716	0.8632	0.6104	0.3934	0.0255
PCB-81	0.00211	0.11990	0.19650	-0.00370	0.03672	0.18115	0.27542	-0.06016
	0.9837	0.2446	0.0550	0.9715	0.7225	0.0773	0.0066	0.5604
PCB-126	0.01952	0.12572	0.22113	0.09502	0.05124	0.25067	0.46057	-0.05439
	0.8503	0.2223	0.0304	0.3571	0.6200	0.0138	<0.0001	0.5986
PCB-169	0.35694	0.32489	0.30091	0.29540	0.25159	0.16267	0.29157	-0.15415
	0.0004	0.0012	0.0029	0.0035	0.0134	0.1133	0.0039	0.1337
total TCDD	-0.01781	0.00892	-0.05049	-0.00127	0.03986	-0.06433	0.04173	-0.11166
	0.8632	0.9313	0.6252	0.9902	0.6998	0.5335	0.6865	0.2788
1,2,3,6,7,8-H6CDD	0.05205	0.08394	0.11780	0.10648	0.10046	0.08391	0.12776	-0.07769
	0.6145	0.4161	0.2530	0.3018	0.3301	0.4163	0.2148	0.4518
total H6CDD	0.04960	0.10416	0.08901	0.14351	0.19697	0.05702	0.10480	0.00176
	0.6313	0.3125	0.3885	0.1630	0.0544	0.5811	0.3096	0.9864
1,2,3,4,6,7,8-H7CDD	0.20958	0.15967	0.24175	0.31256	0.39608	0.06958	0.10509	0.27705
	0.0404	0.1202	0.0176	0.0019	<0.0001	0.5005	0.3082	0.0063
total H7CDD	0.26054	0.19899	0.25959	0.34748	0.41796	0.06914	0.09951	0.16267
	0.0104	0.0519	0.0106	0.0005	<0.0001	0.5033	0.3347	0.1133
OCDD	0.25077	0.23375	0.29379	0.40264	0.41318	0.07557	0.15797	0.16055
	0.0137	0.0219	0.0037	<0.0001	<0.0001	0.4643	0.1242	0.1181

Appendix 3. Continued.

Contaminant	PCB-66/95	PCB-87	PCB-99	PCB-101	PCB-105	PCB-110	PCB-118	PCB-129
PCB-203	0.58673	0.36156	0.81392	0.73900	0.18139	0.43542	0.48238	0.67919
	<0.0001	0.0003	<0.0001	<0.0001	0.0769	<0.0001	<0.0001	<0.0001
PCB-206	0.42686	0.29619	0.72283	0.59275	0.16932	0.36250	0.41641	0.63336
	<0.0001	0.0034	<0.0001	<0.0001	0.0991	0.0003	<0.0001	<0.0001
SPCB	0.59122	0.42297	0.86784	0.75477	0.19449	0.48979	0.50617	0.72382
	<0.0001	<0.0001	<0.0001	<0.0001	0.0576	<0.0001	<0.0001	<0.0001
PCB–77	0.09546	0.21075	-0.00264	0.02407	-0.00903	0.09954	0.02684	-0.01718
	0.3549	0.0393	0.9796	0.8159	0.9304	0.3346	0.7952	0.8680
PCB-81	0.19605	0.14629	0.19984	0.20108	0.16665	0.24040	0.22925	0.15760
	0.0556	0.1550	0.0509	0.0495	0.1046	0.0183	0.0247	0.1252
PCB-126	0.36022	0.48328	0.27413	0.39976	0.25356	0.42033	0.41222	0.46133
	0.0003	<0.0001	0.0069	<0.0001	0.0127	<0.0001	<0.0001	<0.0001
PCB-169	0.27325	0.31886	0.43505	0.33488	0.05292	0.24460	0.24080	0.42125
	0.0071	0.0015	<0.0001	0.0009	0.6086	0.0163	0.0181	<0.0001
total TCDD	0.01008	0.03545	0.11998	0.07127	-0.09482	-0.06179	-0.05383	0.22092
	0.9224	0.7317	0.2443	0.4902	0.3581	0.5498	0.6024	0.0305
1,2,3,6,7,8-H6CDD	0.26100	0.25736	0.33175	0.26647	0.07275	0.28317	0.26191	0.26947
	0.0102	0.0114	0.0010	0.0087	0.4812	0.0052	0.0099	0.0079
total H6CDD	0.25491	0.22132	0.26609	0.22267	0.09582	0.16828	0.17884	0.24479
	0.0122	0.0302	0.0088	0.0292	0.3530	0.1012	0.0813	0.0162
1,2,3,4,6,7,8-H7CDD	0.16996	0.07449	0.31094	0.27544	-0.00853	0.14466	0.14876	0.20414
	0.0978	0.4707	0.0020	0.0066	0.9343	0.1597	0.1480	0.0460
total H7CDD	0.21327	0.10147	0.36278	0.29582	0.00030	0.14298	0.17923	0.24730
	0.0369	0.3253	0.0003	0.0034	0.9977	0.1646	0.0806	0.0151
OCDD	0.22582	0.11898	0.37888	0.35984	0.06467	0.17393	0.23328	0.30846
	0.0270	0.2483	0.0001	0.0003	0.5313	0.0901	0.0222	0.0022

Appendix 3. Continued.

Contaminant	PCB-138	PCB-146	PCB-149	PCB-151	PCB-153	PCB-170/190	PCB-171	PCB-172
PCB-203	0.94039	0.90363	0.48095	0.50773	0.93550	0.92341	0.90188	0.84809
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB-206	0.85069	0.79187	0.33129	0.44007	0.87941	0.94467	0.80697	0.77605
	<0.0001	<0.0001	0.0010	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SPCB	0.97140	0.92548	0.50991	0.53901	0.98064	0.95441	0.90317	0.85806
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB–77	0.0286	-0.03553	0.12505	-0.02784	0.00663	-0.00739	0.00533	-0.08282
	0.8401	0.7311	0.2248	0.7877	0.9489	0.9430	0.9589	0.4224
PCB-81	0.15791	0.13506	0.34516	0.15982	0.12539	0.07896	0.13440	0.07961
	0.1244	0.1895	0.0006	0.1198	0.2235	0.4444	0.1917	0.4407
PCB-126	0.34077	0.34064	0.37955	0.33525	0.31663	0.33663	0.36671	0.31158
	0.0007	0.0007	0.0001	0.0008	0.0017	0.0008	0.0002	0.0020
PCB-169	0.49600	0.45386	0.23650	0.19782	0.51175	0.51250	0.45110	0.42155
	<0.0001	<0.0001	0.0203	0.0534	<0.0001	<0.0001	<0.0001	<0.0001
total TCDD	0.19676	0.15309	-0.08068	-0.08325	0.21875	0.31060	0.20556	0.23902
	0.0547	0.1365	0.4345	0.4200	0.0323	0.0021	0.0445	0.0190
1,2,3,6,7,8-H6CDD	0.34605	0.35078	0.16510	0.26908	0.32353	0.42497	0.39760	0.26199
	0.0006	0.0005	0.1079	0.0080	0.0013	<0.0001	<0.0001	0.0099
total H6CDD	0.29538	0.29938	0.15338	0.20600	0.26646	0.37604	0.36218	0.26135
	0.0035	0.0030	0.1357	0.0440	0.0087	0.0002	0.0003	0.0101
1,2,3,4,6,7,8-H7CDD	0.39687	0.33069	0.04962	0.16498	0.35598	0.49642	0.39715	0.31011
	<0.0001	0.0010	0.6311	0.1082	0.0004	<0.0001	<0.0001	0.0021
total H7CDD	0.43999	0.37806	0.07137	0.19048	0.39821	0.53469	0.43037	0.34817
	<0.0001	0.0001	0.4896	0.0630	<0.0001	<0.0001	<0.0001	0.0005
OCDD	0.48731	0.43229	0.09865	0.26128	0.43734	0.56301	0.47577	0.43984
	<0.0001	<0.0001	0.3389	0.0101	<0.0001	<0.0001	<0.0001	<0.0001

Appendix 3. Continued.

Contaminant	PCB-180	PCB-182/187	PCB-183	PCB-194	PCB-195	PCB-200	PCB-201	PCB-203
PCB-203	0.94574	0.95333	0.98166	0.91119	0.92222	0.85355	0.94246	1.00
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
PCB-206	0.93561	0.82702	0.88735	0.96280	0.87308	0.83436	0.92143	0.91359
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SPCB	0.97645	0.91335	0.98368	0.93630	0.92334	0.89332	0.93200	0.97118
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PCB–77	-0.01482	0.02396	0.02566	-0.04532	-0.02194	-0.02286	-0.04209	0.00557
	0.8860	0.8168	0.8040	0.6610	0.8320	0.8250	0.6839	0.9571
PCB-81	0.09906	0.11087	0.11944	0.05645	0.12058	0.13207	0.06941	0.09991
	0.3369	0.2822	0.2464	0.5848	0.2419	0.1996	0.5016	0.3328
PCB-126	0.30989	0.40232	0.35757	0.29491	0.29243	0.31723	0.38751	0.33303
	0.0021	<0.0001	0.0003	0.0035	0.0038	0.0016	<0.0001	0.0009
PCB-169	0.50905	0.44058	0.49656	0.48730	0.44464	0.45565	0.45267	0.46991
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
total TCDD	0.30525	0.21598	0.24382	0.33216	0.27258	0.17749	0.24171	0.25558
	0.0025	0.0346	0.0167	0.0009	0.0072	0.0836	0.0177	0.0120
1,2,3,6,7,8-H6CDD	0.39642	0.35589	0.34814	0.42250	0.39692	0.42162	0.42447	0.37299
	<0.0001	0.0004	0.0005	<0.0001	<0.0001	<0.0001	<0.0001	0.0002
total H6CDD	0.34592	0.31885	0.29642	0.36119	0.38692	0.39453	0.37132	0.33427
	0.0006	0.0015	0.0034	0.0003	<0.0001	<0.0001	0.0002	0.0009
1,2,3,4,6,7,8-H7CDD	0.46605	0.44340	0.41423	0.48341	0.43801	0.40436	0.49615	0.47528
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
total H7CDD	0.50538	0.47610	0.44858	0.51711	0.47522	0.44967	0.53219	0.50679
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
OCDD	0.51213	0.49562	0.48418	0.53085	0.51571	0.51369	0.55396	0.52989
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Appendix 3. Continued.

Contaminant	PCB-206	∑PCB	PCB-77	PCB-81	PCB-126	PCB-169	total TCDD	1,6,7,8-H6CDD
PCB-203								
PCB-206	1.00							
SPCB	0.93200 <0.0001	1.00						
PCB–77	-0.05321 0.6067	-0.00102 0.9921	1.00					
PCB-81	0.05271 0.6100	0.12474 0.2259	0.32738 0.0011	1.00				
PCB-126	0.29105 0.0040	0.34731 0.0005	0.59388 <0.0001	0.28667 0.0046	1.00			
PCB-169	0.46176 <0.0001	0.51523 <0.0001	0.49787 <0.0001	0.24834 0.0147	0.55133 <0.0001	1.00		
total TCDD	0.26359 0.0095	0.24822 0.0148	0.27107 0.0076	0.18890 0.0653	0.26867 0.0081	0.27770 0.0062	1.00	
1,2,3,6,7,8-H6CDD	0.41666 <0.0001	0.37759 0.0001	0.33321 0.0009	0.16791 0.1020	0.41291 <0.0001	0.44356 <0.0001	0.36345 0.0003	1.00
total H6CDD	0.34762 <0.0001	0.323733 0.0013	0.41404 <0.0001	0.07771 0.4517	0.41018 <0.0001	0.36588 0.0002	0.26826 0.0082	0.74211 <0.0001
1,2,3,4,6,7,8-H7CDD	0.50579 <0.0001	0.43097 <0.0001	0.16108 0.1169	0.05900 0.5680	0.19151 0.0616	0.39177 <0.0001	0.21268 0.0375	0.56077 <0.0001
total H7CDD	0.52999 <0.0001	0.47072 <0.0001	0.12054 0.2421	0.00352 0.9728	0.12808 0.2137	0.34339 0.0006	0.19069 0.0627	0.51174 <0.0001
OCDD	0.55068 <0.0001	0.50488 <0.0001	0.32310 0.0013	0.02542 0.8058	0.23741 0.0199	0.46233 <0.0001	0.23925 0.0189	0.43985 <0.0001

Appendix 3. Continued.

Contaminant	total H6CDD	1,2,3,4,6,7,8-H7CDD	total H7CDD	OCDD	total TCDF	1,2,4,7,8-PCDF	total PCDF	1,2,3,4,7,8-H6CDF
PCB-203								
PCB-206								
SPCB								
PCB–77								
PCB-81								
PCB-126								
PCB-169								
total TCDD								
1,2,3,6,7,8-H6CDD								
total H6CDD	1.00							
1,2,3,4,6,7,8-H7CDD	0.49814 <0.0001	1.00						
total H7CDD	0.55024 <0.0001	0.88906 <0.0001	1.00					
OCDD	0.49426 <0.0001	0.63393 <0.0001	0.68278 <0.0001	1.00				

Appendix 3. Continued.

Contaminant	QCB	HCB	DDE	DDD	DDT	Mirex	Photmirex	β-HCH
total TCDF	-0.01934	0.07567	0.16955	0.18274	0.19212	0.30421	0.14733	0.03817
	0.8516	0.4637	0.0987	0.0747	0.0608	0.0026	0.1520	0.7120
1,2,4,7,8-PCDF	-0.11375	0.00452	0.04429	0.05523	0.19512	0.30960	0.13754	0.15284
	0.2698	0.9651	0.6683	0.5930	0.0568	0.0021	0.1814	0.1371
total PCDF	-0.13496	-0.09531	-0.02673	0.03057	0.17465	0.27872	0.01031	0.21504
	0.1899	0.3556	0.7960	0.7674	0.0888	0.0060	0.9206	0.0354
1,2,3,4,7,8-H6CDF	0.20605	0.05041	0.19071	0.24257	0.07812	0.03256	-0.18550	-0.05314
	0.0440	0.6257	0.0627	0.0173	0.4493	0.7528	0.0704	0.6071
total H6CDF	0.19857	0.05564	0.18596	0.22038	0.13634	0.09551	-0.11440	0.01497
	0.0525	0.5903	0.0697	0.0310	0.1853	0.3546	0.2671	0.8849
1,2,3,4,6,7,8-H7CDF	0.16696	0.01592	0.15252	0.25323	-0.08691	-0.15388	-0.43928	0.05696
	0.1040	0.8777	0.1379	0.0128	0.3998	0.1344	<0.0001	0.5815
total H7CDF	0.22802	0.05251	0.17139	0.26159	-0.04363	-0.11529	-0.43740	0.04601
	0.0255	0.6114	0.0950	0.0100	0.6729	0.2633	<0.0001	0.6562
OCDF	0.16521	0.02428	0.20013	0.29939	-0.02574	0.00963	-0.41919	-0.00728
	0.1077	0.8143	0.0506	0.0030	0.8034	0.9258	<0.0001	0.9439

Appendix 3. Continued.

Contaminant	Oxy-chlordane	trans-Nonachlor	cis-Nonachlor	Heptachlor Epoxide	Dieldrin	PCB-49	PCB-52	PCB-60
total TCDF	0.07719	0.10798	0.10241	0.03479	0.02313	0.24948	0.21697	0.27705
	0.4547	0.2950	0.3208	0.7365	0.8230	0.0142	0.0337	0.0063
1,2,4,7,8-PCDF	0.01845	0.07464	0.12114	-0.04863	0.01620	0.27413	0.23538	0.16267
	0.8584	0.4698	0.2397	0.6380	0.8755	0.0069	0.0210	0.1133
total PCDF	0.00373	0.01524	0.03306	-0.07012	-0.00670	0.20137	0.22946	0.16055
	0.9712	0.8828	0.7492	0.4972	0.9484	0.0491	0.0245	0.1181
1,2,3,4,7,8-H6CDF	0.09516	0.19187	0.26722	0.22749	0.33480	0.17473	0.13722	-0.08163
	0.3564	0.0611	0.0085	0.0258	0.0009	0.0886	0.1825	0.4291
total H6CDF	0.14848	0.21716	0.28472	0.24577	0.33268	0.17043	0.20603	-0.04862
	0.1488	0.0336	0.0049	0.0159	0.0009	0.0969	0.0440	0.6380
1,2,3,4,6,7,8-H7CDF	0.16503	0.19885	0.33280	0.38169	0.41221	0.08570	0.19397	-0.10740
	0.1081	0.0521	0.0009	0.0001	<0.0001	0.4064	0.0583	0.2976
total H7CDF	0.21968	0.23564	0.35171	0.41645	0.42920	0.09967	0.19641	-0.07894
	0.0315	0.0208	0.0004	<0.0001	<0.0001	0.3340	0.0551	0.4446
OCDF	0.11369	0.18632	0.32802	0.25443	0.36553	0.19010	0.26085	0.03193
	0.2701	0.0691	0.0011	0.0124	0.0003	0.0636	0.0103	0.7574

Appendix 3. Continued.

Contaminant	PCB-66/95	PCB-87	PCB-99	PCB-101	PCB-105	PCB-110	PCB-118	PCB-129
total TCDF	0.23124	0.30983	0.07605	0.16768	0.11297	0.21638	0.08454	0.03733
	0.0234	0.0021	0.4615	0.1025	0.2731	0.0342	0.4128	0.7180
1,2,4,7,8-PCDF	0.14992	0.28917	0.21360	0.20619	0.05495	0.16653	0.08000	0.36062
	0.1449	0.0043	0.0367	0.0439	0.5949	0.1049	0.4384	0.0003
total PCDF	0.14164	0.40053	0.28818	0.20922	0.22993	0.24243	0.14724	0.40521
	0.1686	<0.0001	0.0044	0.0408	0.0242	0.0173	0.1523	<0.0001
1,2,3,4,7,8-H6CDF	0.19716	0.06866	0.22554	0.21390	0.14288	0.01975	0.14968	0.20516
	0.0542	0.5062	0.0271	0.0364	0.1649	0.8485	0.1455	0.0449
total H6CDF	0.24980	0.12693	0.25860	0.24203	0.18485	0.08253	0.17265	0.26893
	0.0141	0.2178	0.0110	0.0175	0.0714	0.4241	0.0926	0.0081
1,2,3,4,6,7,8-H7CDF	0.25025	0.17666	0.34118	0.38150	0.13675	0.12676	0.28193	0.29027
	0.0139	0.0851	0.0007	0.0001	0.1840	0.2184	0.0054	0.0041
total H7CDF	0.27071	0.22761	0.37789	0.40021	0.18971	0.15785	0.31082	0.32785
	0.0076	0.0257	0.0001	<0.0001	0.0641	0.1245	0.0021	0.0011
OCDF	0.28571	0.18771	0.36331	0.41061	0.32047	0.23521	0.43542	0.42104
	0.0048	0.0670	0.0003	<0.0001	0.0015	0.0211	<0.0001	<0.0001

Appendix 3. Continued.

Contaminant	PCB-138	PCB-146	PCB-149	PCB-151	PCB-153	PCB-170/190	PCB-171	PCB-172
total TCDF	0.01372	0.06777	0.26292	0.30399	-0.00120	-0.06908	-0.00088	0.03665
	0.8945	0.5118	0.0097	0.0026	0.9908	0.5037	0.9932	0.7230
1,2,4,7,8-PCDF	0.14814	0.21933	0.26842	0.24671	0.17506	0.17857	0.21963	0.26443
	0.1497	0.0318	0.0082	0.0154	0.0880	0.0817	0.0315	0.0092
total PCDF	0.26983	0.30317	0.30874	0.33547	0.26292	0.30866	0.27861	0.30805
	0.0078	0.0027	0.0022	0.0008	0.0097	0.0022	0.0060	0.0023
1,2,3,4,7,8-H6CDF	0.17898	0.23779	0.09561	0.15229	0.18026	0.24255	0.30081	0.21839
	0.0810	0.0197	0.3541	0.1385	0.0788	0.0173	0.0029	0.0325
total H6CDF	0.21435	0.27620	0.14853	0.16663	0.20415	0.24417	0.31372	0.24214
	0.0360	0.0065	0.1487	0.1047	0.0460	0.0165	0.0019	0.0175
1,2,3,4,6,7,8-H7CDF	0.38109	0.38311	0.11657	0.24623	0.35551	0.47705	0.40105	0.33744
	0.0001	0.0001	0.2580	0.0156	0.0004	<0.0001	<0.0001	0.0008
total H7CDF	0.42714	0.42509	0.14099	0.27898	0.40165	0.52083	0.43820	0.37118
	<0.0001	<0.0001	0.1706	0.0059	<0.0001	<0.0001	<0.0001	0.0002
OCDF	0.41898	0.43298	0.29512	0.35778	0.37746	0.49398	0.44502	0.43606
	<0.0001	<0.0001	0.0035	0.0003	0.0001	<0.0001	<0.0001	<0.0001

Appendix 3. Continued.

Contaminant	PCB-180	PCB-182/187	PCB-183	PCB-194	PCB-195	PCB-200	PCB-201	PCB-203
total TCDF	-0.02340	0.05418	0.02063	-0.08121	-0.08069	-0.04821	-0.01918	-0.02319
	0.8210	0.6001	0.8419	0.4315	0.4345	0.6409	0.8529	0.8225
1,2,4,7,8-PCDF	0.19238	0.18910	0.18591	0.18508	0.19298	0.20680	0.20781	0.17046
	0.0604	0.0650	0.0698	0.0710	0.0596	0.0432	0.0422	0.0968
total PCDF	0.28587	0.28349	0.27640	0.28434	0.29459	0.35013	0.33811	0.26518
	0.0048	0.0051	0.0064	0.0050	0.0036	0.0005	0.0008	0.0090
1,2,3,4,7,8-H6CDF	0.26140	0.25356	0.22034	0.25568	0.29661	0.26767	0.27066	0.28449
	0.0101	0.0127	0.0310	0.0119	0.0033	0.0084	0.0076	0.0050
total H6CDF	0.25750	0.27993	0.24864	0.24589	0.32016	0.29395	0.28319	0.29838
	0.0113	0.0057	0.0146	0.0157	0.0015	0.0037	0.0052	0.0031
1,2,3,4,6,7,8-H7CDF	0.44788	0.42360	0.40305	0.46352	0.42836	0.42326	0.48161	0.45434
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
total H7CDF	0.49570	0.45461	0.44775	0.50152	0.46395	0.45951	0.51236	0.49630
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
OCDF	0.44531	0.46772	0.42496	0.47190	0.45534	0.47350	0.52421	0.47453
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Appendix 3. Continued.

Contaminant	PCB-206	∑PCBs	PCB-77	PCB-81	PCB-126	PCB-169	total TCDD	1,6,7,8-H6CDD
total TCDF	-0.10895 0.2907	-0.00610 0.9529	0.05576 0.5894	0.09999 0.3324	0.00280 0.9784	-0.10305 0.3178	-0.11347 0.2710	-0.01774 0.8638
1,2,4,7,8-PCDF	0.13252 0.1981	0.18816 0.0664	0.23118 0.0234	0.20755 0.0425	0.27117 0.0075	0.27634 0.0064	0.13108 0.2030	0.21530 0.0351
total PCDF	0.25029 0.0139	0.29354 0.0037	0.23560 0.0208	0.19365 0.0587	0.34815 0.0005	0.26672 0.0086	0.16094 0.1172	0.28950 0.0042
1,2,3,4,7,8-H6CDF	0.25337 0.0127	0.23117 0.0234	0.26043 0.0104	0.15889 0.1220	0.21466 0.0357	0.32697 0.0011	0.15182 0.1398	0.43529 <0.0001
total H6CDF	0.24213 0.0175	0.25013 0.0140	0.24006 0.0185	0.12392 0.2290	0.19594 0.0557	0.29508 0.0035	0.04710 0.6486	0.38173 0.0001
1,2,3,4,6,7,8-H7CDF	0.48447 <0.0001	0.42581 <0.0001	0.24784 0.0149	0.08227 0.4255	0.31805 0.0016	0.37601 0.0002	0.26716 0.0085	0.48284 <0.0001
total H7CDF	0.51532 <0.0001	0.46960 <0.0001	0.17666 0.0851	0.06988 0.4987	0.32483 0.0012	0.36604 0.0002	0.27395 0.0069	0.43500 <0.0001
OCDF	0.50657 <0.0001	0.45356 <0.0001	0.10007 0.3320	0.23457 0.0214	0.35131 0.0004	0.32957 0.0010	0.26432 0.0093	0.44647 <0.0001

Appendix 3. Continued.

Contaminant	total H6CDD	1,2,3,4,6,7,8-H7CDD	total H7CDD	OCDD	total TCDF	1,2,4,7,8-PCDF	total PCDF	1,2,3,4,7,8-H6CDF
total TCDF	0.05037	-0.12257	-0.02095	-0.09001	1.00			
	0.6260	0.2342	0.8395	0.3832				
1,2,4,7,8-PCDF	0.41618	0.17287	0.25571	0.14240	0.20131	1.00		
	<0.0001	0.0921	0.0119	0.1664	0.0492			
total PCDF	0.49056	0.24272	0.31233	0.22475	0.13468	0.79746	1.00	
	<0.0001	0.0172	0.0019	0.0277	0.1908	<0.0001		
1,2,3,4,7,8-H6CDF	0.58491	0.52627	0.49082	0.37592	-0.11838	0.39001	0.30225	1.00
	<0.0001	<0.0001	<0.0001	0.0002	0.2507	<0.0001	0.0028	
total H6CDF	0.59898	0.42631	0.46335	0.32265	0.00641	0.49317	0.43809	0.87070
	<0.0001	<0.0001	<0.0001	0.0013	0.9505	<0.0001	<0.0001	<0.0001
1,2,3,4,6,7,8-H7CDF	0.56182	0.63657	0.62439	0.62103	-0.20299	0.24025	0.28913	0.63558
	<0.0001	<0.0001	<0.0001	<0.0001	0.0473	0.0184	0.0043	<0.0001
total H7CDF	0.48376	0.61657	0.63690	0.61990	-0.15219	0.20855	0.24914	0.54749
	<0.0001	<0.0001	<0.0001	<0.0001	0.1388	0.0414	0.0144	<0.0001
OCDF	0.38472	0.51465	0.51085	0.53808	-0.16480	0.13533	0.20475	0.49697
	<0.0001	<0.0001	<0.0001	<0.0001	0.1086	0.1886	0.0454	<0.0001

Appendix 3. Continued.

Contaminant	total H6CDF	1,2,3,4,6,7,8-H7CDF	total H7CDF	OCDF
total TCDF				
1,2,4,7,8-PCDF				
total PCDF				
1,2,3,4,7,8-H6CDF				
total H6CDF	1.00			
1,2,3,4,6,7,8-H7CDF	0.57646 <0.0001	1.00		
total H7CDF	0.49749 <0.0001	0.93621 <0.0001	1.00	
OCDF	0.43503 <0.0001	0.67089 <0.0001	0.69066 <0.0001	1.00

APPENDIX 4

Appendix 4. Arithmetic mean mercury, lead, and cadmium concentrations in the European otter (Lutra lutra) tissues. Reported concentrations are expressed as μg g^{-1} in either wet (fresh) or dry mass. Conversion between wet and dry mass was done using an arithmetic mean of 71% moisture for both liver and kidney tissue derived from this study. Values in bold are actual values reported.

		Wet Mass		Dry Mass		
Location	n	Mean	Range	Mean	Range	Reference
Mercury (**Liver**)						
Scotland	1	31.0		**107**		Kruuk et al., 1993
Sweden	8	**16.5**	**4.1-30.7**	56.9	14.1-105.9	Olsson and Sandegren, 1991
Czech Republic	5	**14.4**	**nd-55.6**	48.0	nd-185.3	Gutleb et al., 1998
Doñana Spain	5	**8.28**[b]	**3.92-17.5**	28.6	13.5-60.3	Hernandez et al., 1985
Elbe River, Germany	1	**7.9**		**28.1**		Röchert, 1989
Finland Adult Male	9	**5.50**	**(1.13)**	19.0		Hyvärinen et al., 2003
England	19	**5.37**	**1.20-20.5**	18.5	4.14-70.7	Mason et al., 1986b
Argyll	18	5.37, 4.09[b]		**18.5, 14.1**[b]	**(12.7, 2.3**[c]**)**	Kruuk et al., 1993 and 1997
N. England/Wales	3	5.22		**18.0**	**(24.7)**	Kruuk et al., 1993
Orkney Islands	14	**4.70**	**1.00-20.3**	16.2	3.45-70.0	Mason and Reynolds, 1988
Orkney	14	4.41, 3.83[b]		**15.2, 13.2**[b]	**(8.50, 1.8**[c]**)**	Kruuk et al., 1993 and 1997
Northern Scotland	10	4.41, 2.87[b]		**15.2, 9.9**[b]	**(12.9, 3.1**[c]**)**	Kruuk et al., 1993 and 1997
Scotland	2	**4.28**	**4.23-4.33**	14.8	14.6-14.9	Mason, 1988
Speyside/Inverness	10	4.09, 2.78[b]		**14.1, 9.6**[b]	**(11.2, 2.8**[c]**)**	Kruuk et al., 1993 and 1997
United Kingdom	112	**4.04**	**0.11-13.4**	**13.3**	**0.33-44.7**	Kruuk et al., 1993 and 1997
Finland Adult	10	**3.80**	**(0.49)**	13.1		Hyvärinen et al., 2003
Shetland	86	3.71	nd-18.9	**12.8**	**nd-65.0**	Kruuk and Conroy, 1991
Shetland	14	3.57, 3.19[b]		**12.3, 11.0**[b]	**(5.39, 1.6**[c]**)**	Kruuk et al., 1993 and 1997
Grampian	33	2.42, 1.71[b]		**8.35, 5.9**[b]	**(6.73, 2.6**[c]**)**	Kruuk et al., 1993 and 1997
Denmark	69	**2.30**	**0.03-12.37**	7.93	0.10-42.7	Mason and Madsen, 1992
Ireland	1	**2.19**		7.55		Mason, 1988
SW England	3	**1.88**	**0.24-3.50**	6.48	0.83-12.1	Mason, 1988
Dumfries/Galloway	7, 10[a]	1.53, 3.51[b]		**5.29, 12.1**[b]	**(11.4, 2.0**[c]**)**	Kruuk et al., 1993 and 1997

Appendix 4. Continued.

Location	n	Wet Mass Mean	Wet Mass Range	Dry Mass Mean	Dry Mass Range	Reference
Mercury (**Liver**)						
Finland Juvenile	12	**1.20**	**(0.28)**	4.14		Hyvärinen et al., 2003
Austria	15	**1.01**	**nd-1.84**	3.48	nd-6.34	Gutleb et al., 1998
Netherlands	4	**0.68**	**0.30-0.90**	**2.53**	**1.20-3.40**	Broekhuizen, 1989
Greece	1	**0.68**		2.34		Gaethlich and Mason, 1986
Hungary	7	**0.65**	**0.02-2.64**	2.24	0.07-9.10	Gutleb et al., 1998
Spain	19	0.29	nd-0.81	**0.99**	**nd-2.80**	Ruiz-Olmo et al., 1998
Iisalmi, Finland	1	**0.28**		0.97		Skarén and Kumpulainen, 1986
East Anglia, UK	1	**0.17**		0.59		Mason, 1988
Mercury (**Kidney**)						
Greece	1	**0.21**		0.72		Gaethlich and Mason, 1986
England	16	**2.27**	**1.35-6.79**	7.83	4.66-23.4	Mason et al., 1986b
Orkney Islands	9	**2.10**	**1.50-3.00**	7.24	5.17-10.3	Mason and Reynolds, 1988
Ireland	1	**1.45**		5.00		Mason, 1988
Finland Adult Male	9	**1.40**	**(0.24)**	4.83		Hyvärinen et al., 2003
Finland Adult	10	**1.30**	**(0.21)**	4.48		Hyvärinen et al., 2003
East Anglia, UK	2	**1.06**	**0.53-1.59**	3.66	1.83-5.48	Jefferies and Hanson, 1988
SW England	3	**1.01**	**0.11-2.02**	3.48	0.38-7.00	Mason, 1988
Scotland	2	**0.89**	**0.76-1.02**	3.07	2.62-3.52	Mason, 1988
Netherlands	4	**0.68**	**0.20-1.30**	**3.03**	**0.90-5.6**	Broekhuizen, 1989
Austria	15	**0.68**	**nd-2.10**	2.34	nd-7.24	Gutleb et al., 1998
Hungary	7	**0.44**	**0.04-0.87**	1.52	0.14-3.00	Gutleb et al., 1998
Finland Juvenile	12	**0.40**	**(0.10)**	1.38		Hyvärinen et al., 2003
Czech Republic	2	**0.32**	**0.25-0.38**	1.10	0.86-1.31	Gutleb et al., 1998
East Anglia, UK	1	**0.08**		0.28		Mason, 1988
Mercury (**Hair**)						
Finland Adult Male	9			**29.5**	**(3.53)**	Hyvärinen et al., 2003

Appendix 4. Continued.

Location	n	Wet Mass Mean	Wet Mass Range	Dry Mass Mean	Dry Mass Range	Reference
Mercury (**Hair**)						
Sweden	20			**26.5**	**2.80-96.0**	Olsson and Sandegren, 1991
Finland Adult	10			**20.0**	**(2.45)**	Hyvärinen et al., 2003
Orkney Islands	5			**18.9**	**9.50-29.5**	Mason and Reynolds, 1988
England	25			**18.75**	**1.3-85.1**	Mason et al., 1986b
Finland Juvenile	12			**5.90**	**(1.17)**	Hyvärinen et al., 2003
Greece	1			**4.76**		Gaethlich and Mason, 1986
Denmark	52			**2.38**	**nd-13.49**	Madsen and Mason, 1987
Lead (**Liver**)						
Greece	1	**2.69**		8.28		Gaethlich and Mason, 1986
England	19	**2.20**	**nd-5.86**	7.59	nd-20.2	Mason et al., 1986b
Orkney Islands	14	**1.74**	**0.13-3.65**	6.00	0.45-12.6	Mason and Reynolds, 1988
Doñana Spain	5	**0.73**	**0.69-0.78**	2.52	2.38-2.69	Hernandez et al., 1985
Ireland	1	**0.56**		1.93		Mason, 1988
SW England	3	**0.54**	**0.31-0.72**	1.86	1.07-2.48	Mason, 1988
Scotland	2	**0.45**	**0.09-0.81**	1.55	0.31-2.79	Mason, 1988
East Anglia	1	**0.42**		1.45		Mason, 1988
Hungary	7	0.24	0.10-0.64	**0.83**	**0.33-2.20**	Gutleb et al., 1998
Netherlands	4	**0.13**	**0.05-0.20**	**0.43**	**0.20-0.70**	Broekhuizen, 1989
Austria	15	0.11	0.003-0.34	**0.37**	**0.01-1.17**	Gutleb et al., 1998
Czech Republic	5	0.11	0.02-0.23	**0.39**	**0.08-0.80**	Gutleb et al., 1998
Elbe River, Germany	1	**0.10**		**0.36**		Röchert, 1989
Austria	4	0.05	0.01-0.10	**0.16**	**0.03-0.36**	Gutleb, 1992
Spain	19	0.03	nd-0.10	**0.09**	**nd-0.34**	Ruiz-Olmo et al., 1998
Lead (**Kidney**)						
Greece	1	**8.40**		29.0		Gaethlich and Mason, 1986
Orkney Islands	9	**2.34**	**1.38-3.80**	8.07	4.76-13.1	Mason and Reynolds, 1988

Appendix 4. Continued.

Location	n	Wet Mass Mean	Wet Mass Range	Dry Mass Mean	Dry Mass Range	Reference
Lead (**Kidney**)						
England	16	**1.97**	**nd-3.84**	6.79	nd-13.2	Mason et al., 1986b
East Anglia	1	**0.77**		2.66		Mason, 1988
SW England	3	**0.65**	**0.41-0.88**	2.24	1.37-2.93	Mason, 1988
Scotland	2	**0.50**	**0.11-0.89**	1.72	0.38-3.07	Mason, 1988
Elbe River, Germany	1	**0.34**		**1.30**		Röchert, 1989
Hungary	7	0.32	0.05-0.69	**1.12**	**0.17-2.39**	Gutleb et al., 1998
Ireland	1	**0.23**		0.79		Mason, 1988
Austria	15	0.20	0.04-1.10	**0.69**	**0.15-3.78**	Gutleb et al., 1998
Netherlands	4	**0.16**	**0.07-0.30**	**0.73**	**0.30-1.30**	Broekhuizen, 1989
Austria	4	0.11	0.01-0.21	**0.39**	**0.04-0.73**	Gutleb, 1992
Czech Republic	2	0.07	0.01-0.13	**0.23**	**0.02-0.44**	Gutleb et al., 1998
Lead (**Hair**)						
Greece	1			**19.05**		Gaethlich and Mason, 1986
England	25			**13.05**	**nd-88.5**	Mason et al., 1986b
Orkney Islands	9			**2.70**	**0.05-10**	Mason and Reynolds, 1988
Denmark	52			**1.28**	**nd-7.40**	Madsen and Mason, 1987
Cadmium (**Liver**)						
Scotland	2	**0.52**	**0.26-0.77**	1.79	0.90-2.66	Mason, 1988
Czech Republic	5	0.44	0.05-1.57	**1.51**	**0.16-5.42**	Gutleb et al., 1998
SW England	3	**0.28**	**0.15-0.44**	0.97	0.52-1.52	Mason, 1988
England	19	**0.27**	**nd-0.54**	0.93	nd-1.86	Mason et al., 1986b
Orkney Islands	14	**0.22**	**nd-0.39**	0.76	nd-1.34	Mason and Reynolds, 1988
Doñana Spain	5	0.18	0.12-0.27	**0.63**	**0.43-0.93**	Hernandez et al., 1985
Greece	1	0.18		**0.63**		Gaethlich and Mason, 1986
East Anglia	1	**0.18**		0.62		Mason, 1988

Appendix 4. Continued.

Location	n	Wet Mass Mean	Wet Mass Range	Dry Mass Mean	Dry Mass Range	Reference
Cadmium (Liver)						
Austria	15	0.10	nd-0.25	**0.36**	**nd-0.87**	Gutleb et al., 1998
Hungary	7	0.09	0.01-0.14	**0.31**	**0.05-0.49**	Gutleb et al., 1998
Spain	19	0.01	nd-0.06	**0.04**	**nd-0.22**	Ruiz-Olmo et al., 1998
Cadmium (**Kidney**)						
Scotland	2	**1.14**	**0.28-1.99**	3.93	0.97-6.86	Mason, 1988
England	16	**0.60**	**0.08-2.18**	2.07	0.28-7.52	Mason et al., 1986b
Ireland	1	**0.57**		1.97		Mason, 1988
Shetland	38	0.51	nd-1.97	**1.76**	**nd-6.8**	Kruuk and Conroy, 1991
East Anglia	1	**0.39**		1.34		Mason, 1988
Orkney Islands	9	**0.34**	**0.08-0.56**	1.17	0.28-1.93	Mason and Reynolds, 1988
SW England	3	**0.34**	**0.22-0.56**	1.17	0.78-1.93	Mason, 1988
Austria	15	0.26	0.01-1.33	**0.91**	**0.03-4.6**	Gutleb et al., 1998
Ireland	1	**0.23**		0.79		Mason, 1988
Netherlands	4	**0.16**	**0.1-0.2**	0.55	0.34-0.69	Broekhuizen, 1989
Hungary	7	0.15	0.05-0.26	**0.51**	**0.17-0.89**	Gutleb et al., 1998
Netherlands	4	**0.13**	**0.10-0.20**	0.45	0.34-0.69	Broekhuizen, 1989
Greece	1	**0.09**		0.31		Gaethlich and Mason, 1986
Czech Republic	2	0.09	0.08-0.10	**0.32**	**0.28-0.36**	Gutleb et al., 1998
Elbe River, Germany	1	**0.08**		0.28		Röchert, 1989
Elbe River, Germany	1	**0.03**		0.10		Röchert, 1989
Cadmium (**Hair**)						
England	25			**1.12**	**nd-15.9**	Mason et al., 1986b
Denmark	52			**0.77**	**nd -2.67**	Madsen and Mason, 1987
Greece	1			**0.23**		Gaethlich and Mason, 1986
Finland Adult	11			**0.038**	**(0.036)**	Hyvärinen et al., 2003
Finland Adult Male	11			**0.036**	**(0.025)**	Hyvärinen et al., 2003

Appendix 4. Continued.

Location	n	Wet Mass Mean	Wet Mass Range	Dry Mass Mean	Dry Mass Range	Reference
Cadmium (**Hair**)						
Finland Juvenile	17			**0.005**	**(0.004)**	Hyvärinen et al., 2003

[a] n for Kruuk et al., 1997.
[b] Geometric mean.
[c] Standard deviation of geometric mean.
Range value in ( ) represents the standard deviation reported instead of range.

APPENDIX 5

Appendix 5. Arithmetic mean DDE concentrations in the European otter (Lutra lutra) tissues. Reported concentrations are μg g^{-1} in either wet (fresh mass) or lipid mass. Conversion between lipid and wet mass was done using an arithmetic mean of 3.8% lipid for liver and 2.0% for muscle derived from Jefferies and Hanson 1987, Skarén 1988, Lopez-Martin and Ruiz-Olmo 1996, and Sjöåsen et al. 1997. Values in bold are actual values reported.

		Wet Mass		Lipid Mass		
Location	n	Mean	Range	Mean	Range	Reference
Liver						
Doñana Spain	5	**2.85**	**1.97-4.12**	74.1	51.2-107	Hernandez et al., 1985
Finland	1	0.02		**0.40**		Skarén and Kumpulainen, 1986
Orkney Islands	1	0.01		**0.30**		Mason et al., 1986a
Scotland	3	2.28	0.13-4.45	**59.4**	**3.50-115.7**	Mason et al., 1986a
East Anglia	2	2.75	1.66-3.85	**71.6**	**43.1-100**	Mason et al., 1986a
Wales	3	0.48	0.11-0.33	**12.6**	**2.80-8.60**	Mason et al., 1986a
United Kingdom	26					Jefferies and Hanson, 1987
Central Finland	10	**0.07**	**0.02-0.23**	**4.07**	**0.70-28.5**	Skarén, 1988
Orkney Islands	14	0.04	nd-0.28	**1.02**	**nd-7.18**	Mason and Reynolds, 1988
Shetland	73	**0.67**	**nd-7.80**	17.4	nd-203	Kruuk and Conroy, 1991
Ireland: rural	28[a]	0.15	0.001-0.99	**3.96**	**0.05-25.7**	Mason and O'Sullivan, 1992
Ireland: Cork City	5	1.65	0.17-7.31	**42.9**	**4.53-190**	Mason and O'Sullivan, 1992
Ireland	11	0.55	0.02-1.64	**14.4**	**0.60-42.6**	Mason and O'Sullivan, 1993
Denmark	73[b]	0.11	0.005-3.78	**2.88**	**0.12-98.4**	Mason and Madsen, 1993
Shetland	14	**0.11**	**(0.13)**	2.86		Kruuk et al., 1993
Orkney	14	**0.11**	**(0.09)**	2.86		Kruuk et al., 1993
Northern Scotland	10	**0.06**	**(0.08)**	1.56		Kruuk et al., 1993
Argyll	18	**0.14**	**(0.21)**	3.64		Kruuk et al., 1993
Dumfries/Gallowaya	7	**0.16**	**(0.12)**	4.16		Kruuk et al., 1993
Speyside/Inverness	10	**0.19**	**(0.17)**	4.94		Kruuk et al., 1993
Grampian	33	**0.50**	**(0.50)**	13.0		Kruuk et al., 1993
N. England/Wales	1	0.01		**0.26**		Kruuk et al., 1993
SW England	20	0.93	0.05-6.19	**24.1**	**1.40-161**	Mason and Macdonald, 1994

Appendix 5. Continued.

Location	n	Wet Mass Mean	Wet Mass Range	Lipid Mass Mean	Lipid Mass Range	Reference
Liver						
Scotland	116	**0.12***	**nd-2.81**	**4.00***	**nd-117**	Kruuk and Conroy, 1996
Western France	23	0.14	(**0.13**)	**3.63**	**(3.40)**	Tans et al., 1996
Spain	41	0.97	0.01-5.65	**25.1**	**0.27-147**	Ruiz-Olmo et al. 1998
SW England	56	**0.36**	**0.01-2.40**	9.36	0.26-62.4	Simpson et al., 2000
Scotland	62	**0.95**	**nd-8.52**	**28.1**	**nd-435**	Jefferies and Hanson, 2000
Wales	17	**1.05**	**nd-4.50**	**27.3**	**nd-139**	Jefferies and Hanson, 2000
England	43	**1.18**	**nd-19.5**	**27.0**	**nd-332**	Jefferies and Hanson, 2000
Muscle						
Sweden	53	2.40	0.09-19.4	**120**	**4.70-970**	Sandegren et al., 1980
Norway	23	0.34	0.03-0.60	**17.0**	**1.60-30.0**	Sandegren et al., 1980
North Sweden	24	1.04	0.15-3.40	**52.0**	**7.40-170**	Olsson et al., 1981
South Sweden	29	3.56	0.24-19.4	**178**	**12.0-970**	Olsson et al., 1981
Doñana Spain	4	**0.58***	**0.39-0.87**	29.0*	19.5-43.5	Hernandez et al., 1985
Orkney Islands	1	tr		**tr**		Mason et al., 1986a
Scotland	1	nd		**nd**		Mason et al., 1986a
East Anglia	4	0.62	nd-1.70	**30.9**	**nd-85.0**	Mason et al., 1986a
Wales	6	0.16	nd-0.26	**7.93**	**nd-13.2**	Mason et al., 1986a
SW England	2	0.09	0.06-0.14	**4.73**	**3.10-7.10**	Mason et al., 1986a
Finland	1	0.005		**0.27**		Skarén and Kumpulainen, 1986
Central Finland	1	0.03		**0.50**		Skarén, 1988
Germany[a]	1	nd		**nd**		Röchert, 1989
Western France	23	0.04		**2.17**	**(2.20)**	Tans et al., 1996
Spain	6	0.07*	0.009-0.49	**3.32***	**0.44-24.7**	Lopez-Martin and Ruiz-Olmo,
Sweden Venta/Gauja	8	0.004[c]	0.001-0.02	**0.22**[c]	**0.03-0.80**	Sjöåsen et al., 1997
Spain	41	0.19	0.002-1.05	**9.32**	**0.12-52.3**	Ruiz-Olmo et al. 1998

Appendix 5. Continued.

Location	n	Wet Mass Mean	Wet Mass Range	Lipid Mass Mean	Lipid Mass Range	Reference
Muscle						
Northern Sweden						
Trauma 1970s	21	0.05**[c]	0.01-0.30	**2.30**[**c]	**0.30-15**	Roos et al., 2001
Trauma 1980s	14	0.02**[c]	0.004-0.06	**1.20**[**c]	**0.20-3.00**	Roos et al., 2001
Trauma 1990-94	29	0.002**[c]	0.001-0.05	**0.10**[**c]	**0.03-2.60**	Roos et al., 2001
Unknown 1970s	5	0.11**[c]	0.02-0.54	**5.50**[**c]	**1.20-27**	Roos et al., 2001
Unknown 1980s	2	0.02**[c]	0.016-0.02	**0.90**[**c]	**0.80-1.00**	Roos et al., 2001
Southern Sweden						
Trauma 1970s	19	0.05**[c]	0.01-0.19	**2.60**[**c]	**0.60-9.40**	Roos et al., 2001
Trauma 1980s	7	0.02**[c]	0.004-0.14	**1.20**[**c]	**0.20-7.10**	Roos et al., 2001
Trauma 1990-94	10	0.01**[c]	0.002-0.11	**0.70**[**c]	**0.10-5.50**	Roos et al., 2001
Unknown 1970	12	0.07**[c]	ND-0.48	**3.50**[**c]	**ND-24**	Roos et al., 2001
Unknown 1980	2	0.02**[c]	0.01-0.02	**0.80**[**c]	**0.60-1.10**	Roos et al., 2001
Unknown 1990-94	4	0.02**[c]	0.002-0.13	**0.80**[**c]	**0.10-6.40**	Roos et al., 2001

[a] 25 liver and 3 muscle samples.
[b] 71 liver and 2 muscle samples.
[c] Total DDTs were used.
DDE means with * are geometric and ** are median. nd = not detected. tr = trace.
Range value in ( ) represents the standard deviation reported instead of the range.

APPENDIX 6

Appendix 6. Arithmetic mean dieldrin concentrations in the European otter (Lutra lutra) tissues. Reported concentrations are μg g^{-1} in either wet (fresh) or lipid mass. Conversion between lipid and wet mass was done using an arithmetic mean of 3.8% lipid for liver and 2.0% for muscle from Jefferies and Hanson 1987, Skarén 1988, Lopez-Martin and Ruiz-Olmo 1996, and Sjöåsen et al. 1997. Values in bold are actual reported values.

		Wet Mass		Lipid Mass		
Location	n	Mean	Range	Mean	Range	Reference
Liver						
Doñana Spain	5	**0.013***	**nd-0.02**	0.34*	nd-0.52	Hernandez et al., 1985
Orkney Islands	6	0.005	nd-0.01	**0.12**	**nd-0.3**	Mason et al., 1986a
Scotland	3	0.84	tr-2.55	**22.1**	**tr-66.4**	Mason et al., 1986a
East Anglia	3	1.20	0.30-2.27	**31.6**	**7.9-59**	Mason et al., 1986a
Wales	3	0.26	nd-0.57	**6.8**	**nd-14.7**	Mason et al., 1986a
United Kingdom	26		**0.08-2.45**		**2.14-65.4**	Jefferies and Hanson, 1987
Central Finland	10	**0.007**	**0.0006-0.02**	**0.24**	**0.01-0.5**	Skarén, 1988
Orkney Islands	14	0.01	nd-0.09	**0.27**	**nd-2.31**	Mason and Reynolds, 1988
Shetland	72	**0.17**	**nd-1.0**	4.47	nd-26.0	Kruuk and Conroy, 1991
Ireland: rural	28[a]	0.06	0.003-0.62	**1.62**	**0.08-16.01**	Mason and O'Sullivan, 1992
Ireland: Cork City	5	2.97	0.04-14.3	**77.1**	**1.16-370**	Mason and O'Sullivan, 1992
Ireland	11	0.28	0.01-0.57	**7.34**	**0.37-14.7**	Mason and O'Sullivan, 1993
Denmark	73[b]	0.04	0.002-0.48	**1.1**	**0.04-12.6**	Mason and Madsen, 1993
Shetland	14	**0.07**	**(0.06)**	1.84		Kruuk et al., 1993
Orkney	14	**0.10**	**(0.04)**	2.63		Kruuk et al., 1993
Northern Scotland	10	**0.06**	**(0.03)**	1.58		Kruuk et al., 1993
Argyll	18	**0.09**	**(0.05)**	2.37		Kruuk et al., 1993
Dumfries/Gallowaya	7	**0.11**	**(0.04)**	2.89		Kruuk et al., 1993
Speyside/Inverness	10	**0.09**	**(0.04)**	2.37		Kruuk et al., 1993
Grampian	33	**0.11**	**(0.06)**	2.89		Kruuk et al., 1993
N. England/Wales	1	**0.20**	**(0.07)**	5.26		Kruuk et al., 1993
SW England	20	0.41	0.0004-2.78	**10.7**	**0.01-72.4**	Mason and Macdonald, 1994
Scotland	116	**0.084**	**0.024-0.28**	**3.99**	**nd-117**	Kruuk and Conroy, 1996

Appendix 6. Continued.

Location	n	Wet Mass Mean	Wet Mass Range	Lipid Mass Mean	Lipid Mass Range	Reference
Liver						
Western France	23	0.06		**1.57**	**(2.56)**	Tans et al., 1996
Spain	41	0.01	nd-0.06	**0.27**	**nd-1.64**	Ruiz-Olmo et al., 1998
SW England	56	**0.30**	**0.013-2.80**	7.80	0.34-72.8	Simpson et al., 2000
Scotland	62	**0.25**	**nd-2.45**	**6.94**	**nd-50.6**	Jefferies and Hanson, 2000
Wales	17	**0.36**	**nd-1.00**	**8.87**	**nd-30.9**	Jefferies and Hanson, 2000
England	43	**0.83**	**nd-14.0**	**18.7**	**nd-237**	Jefferies and Hanson, 2000
Muscle						
Doñana Spain	5	**0.014***	**nd-0.10**	0.70*	nd-5.00	Hernandez et al., 1985
Orkney Islands	1	nd		**nd**		Mason et al., 1986a
Scotland	1	nd		**nd**		Mason et al., 1986a
East Anglia	4	0.30	0.07-0.58	**15.1**	**3.30-29**	Mason et al., 1986a
Wales	6	0.06	4 nd-0.07	**3.00**	**4 nd-3.7**	Mason et al., 1986a
SW England	2	0.17	1 nd-0.37	**8.40**	**1 nd-18.7**	Mason et al., 1986a
Central Finland	1	**0.004**		**0.01**		Skarén, 1988
Western France	32	0.02		**0.75**	**(1.45)**	Tans et al., 1996
Spain	41	0.01	nd-0.03	**0.26**	**nd-1.53**	Ruiz-Olmo et al., 1998

[a] 25 liver and 3 muscle samples.

[b] 71 liver and 2 muscle samples.

Dieldrin means with * are geometric. nd = dot detected. tr = trace.

Range value in ( ) represents the standard deviation reported instead of the range.

APPENDIX 7

Appendix 7. Arithmetic mean total PCB concentrations in the European otter (Lutra lutra) tissues. Reported concentrations are in μg g^{-1} as either wet (fresh) or lipid mass. Conversion between lipid and wet mass was done using an arithmetic mean of 3.8% lipid for liver and 2.0% for muscle derived from Jefferies and Hanson 1987, Skarén 1988, Lopez-Martin and Ruiz-Olmo 1996, and Sjöåsen et al. 1997. Values in bold are actual values reported.

		Wet Mass		Lipid Mass		
Location	n	Mean	Range	Mean	Range	Reference
Liver						
Doñana Spain[a]	5	**2.45***	**2.40-2.45**	63.7*	62.4-63.7	Hernandez et al., 1985
Finland[a]	1	0.07		**1.90**		Skarén and Kumpulainen, 1986
Orkney Islands[a]	7	0.79	6 nd-0.79	**20.5**	**6 nd-20.5**	Mason et al., 1986a
Scotland[a]	3	0.23	2 nd-0.23	**6.00**	**2 nd-6.00**	Mason et al., 1986a
East Anglia[a]	3	5.04	0.55-8.92	**131**	**14.2-232**	Mason et al., 1986a
Wales[a]	3	0.20	0.11-0.33	**5.30**	**2.80-8.60**	Mason et al., 1986a
United Kingdom[a]	26		**0.07-4.49**		1.82-118	Jefferies and Hanson, 1987
Central Finland[a]	10	**0.72**	**0.08-2.50**	**29.1**	**5.70-150**	Skarén, 1988
Orkney Islands[a]	14	0.08	nd-0.79	**2.01**	**nd-20.50**	Mason and Reynolds, 1988
Shetland[a]	73	**5.50**	**nd-46.0**	143	nd-1196	Kruuk and Conroy, 1991
Ireland: rural[a]	28[c]	0.58	0.01-4.73	**15.2**	**0.18-123**	Mason and O'Sullivan, 1992
Ireland: Cork City[a]	5	4.54	0.60-17.5	**118**	**15.6-455**	Mason and O'Sullivan, 1992
Ireland[a]	11	1.87	0.10-8.31	**38.7**	**2.00-216**	Mason and O'Sullivan, 1993
Denmark[a]	73[d]	0.64*	0.02-10.6	**16.8***	**0.47-276**	Mason and Madsen, 1993
Shetland[a]	14	**3.50**	**(4.00)**	91.0		Kruuk and Conroy, 1993
Orkney[a]	14	**0.57**	**(0.51)**	14.8		Kruuk and Conroy, 1993
Northern Scotland[a]	10	**0.83**	**(1.21)**	21.6		Kruuk and Conroy, 1993
Argyll[a]	18	**1.19**	**(1.85)**	30.9		Kruuk and Conroy, 1993
Dumfries/Gallowaya[a]	7	**1.64**	**(1.49)**	42.6		Kruuk and Conroy, 1993
Speyside/Inverness[a]	10	**1.02**	**(0.77)**	26.5		Kruuk and Conroy, 1993
Grampian[a]	33	**1.11**	**(1.15)**	28.6		Kruuk and Conroy, 1993
N. England/Wales[a]	3	**2.60**	**(0.93)**	67.6		Kruuk and Conroy, 1993
SW England[a]	20	2.30	0-20.1	**59.9**	**0.01-522**	Mason and Macdonald, 1994

Appendix 7. Continued.

Location	n	Wet Mass		Lipid Mass		Reference
		Mean	Range	Mean	Range	
Liver						
Scotland[a]	116	**0.80**	**nd-14.4**	**27.4**	**nd-596**	Kruuk and Conroy, 1996
Western France[b]	23	1.46		**37.9**	**(32.9)**	Tans et al., 1996
Spain[b]	41	4.12	0.11-37.5	**107**	**2.79-975**	Ruiz-Olmo et al. 1998
The Netherlands[b]	5	1.34	0.17-8.54	**34.8**	**4.43-222**	Leonards et al., 1998
SW England[b]	56	**0.37**	**nd-2.23**	9.62	nd-58.0	Simpson et al., 2000
Scotland[a]	62	**2.24**	**nd-19.4**	**76.4**	**nd-985**	Jefferies and Hanson, 2000
Wales[a]	17	**1.41**	**nd-4.07**	**34.5**	**nd-128**	Jefferies and Hanson, 2000
England[a]	43	**1.75**	**nd-14.9**	**54.1**	**nd-507**	Jefferies and Hanson, 2000
Muscle						
Sweden[a]	53	2.40	0.09-19.4	**120**	**4.70-970**	Sandegren et al., 1980
Norway[a]	23	0.34	0.03-0.60	**17.0**	**1.60-30.0**	Sandegren et al., 1980
North Sweden[a]	24	1.04	0.15-3.40	**52.0**	**7.40-170**	Olsson et al., 1981
South Sweden[a]	29	3.56	0.25-19.4	**178**	**12.0-970**	Olsson et al., 1981
Doñana Spain[a]	4	**1.03***	**0.66-1.63**	51.5*	33.0-81.5	Hernandez et al., 1985
Scotland[a]	1	0.14		**6.90**		Mason et al., 1986a
East Anglia[a]	4	2.18	0.07-6.00	**109**	**3.60-300**	Mason et al., 1986a
Wales[a]	6	0.55	nd-2.26	**27.6**	**nd-113**	Mason et al., 1986a
SW England[a]	2	1.34	0.50-2.18	**67.0**	**25.0-109**	Mason et al., 1986a
Norfolk England[a]	2	3.50	1.04-5.98	**175**	**51.8-299**	Keymer et al., 1988
Central Finland[a]	1	**0.37**		**6.90**		Skarén, 1988
Germany[a]	1	0.68		**34.0**		Röchert, 1989
Western France[b]	32	0.52		**26.2**	**(21.7)**	Tans et al., 1996
Spain[b]	6	0.40	0.14-1.29	**20.0**	**7.21-64.3**	Lopez-Martin and Ruiz-Olmo,
Spain[b]	41	1.57	0.03-20.1	**78.3**	**1.49-1006**	Ruiz-Olmo et al., 1998
Sweden Venta/Gauja[a]	8	**0.12**	**0.006-0.52**	**2.31**	**0.42-10.0**	Sjöåsen et al., 1997

Appendix 7. Continued.

Location	n	Wet Mass Mean	Wet Mass Range	Lipid Mass Mean	Lipid Mass Range	Reference
Muscle						
Northern Sweden						
Trauma 1970s[b]	21	0.54**	0.09-2.80	**27.0****	**4.70-140**	Roos et al., 2001
Trauma 1980s[b]	14	0.44**	0.07-5.80	**22.0****	**3.30-290**	Roos et al., 2001
Trauma 1990-94[b]	29	0.15**	0.15-1.50	**7.70****	**0.60-75.0**	Roos et al., 2001
Unknown 1970s[b]	5	2.20**	0.84-3.40	**110****	**42.0-170**	Roos et al., 2001
Unknown 1980s[b]	2	1.46**	0.52-2.40	**73.0****	**26.0-120**	Roos et al., 2001
Southern Sweden						
Trauma 1970s[b]	19	1.34**	0.10-4.34	**67.0****	**5.00-217**	Roos et al., 2001
Trauma 1980s[b]	7	0.66**	0.18-4.74	**33.0****	**9.00-237**	Roos et al., 2001
Trauma 1990-94[b]	10	0.58**	0.14-2.98	**29.0****	**7.00-149**	Roos et al., 2001
Unknown 1970s[b]	12	4.80**	0.11-19.4	**240****	**5.70-970**	Roos et al., 2001
Unknown 1980s[b]	2	3.68**	1.34-6.00	**184****	**67.0-300**	Roos et al., 2001
Unknown 1990-94[b]	4	1.84**	0.88-17.2	**92.0****	**44.0-860**	Roos et al., 2001

[a] Total PCBs calculated based on Aroclor or Clophen standard.
[b] Total PCBs based on summation of the individual congeners analyzed.
[c] 25 liver and 3 muscle samples.
[d] 71 liver and 2 muscle samples.
PCB mean concentrations with * are geometric and ** are median. nd = not detected.
Range value ( ) represents the standard deviation reported instead of the range.