

REPORTS

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Materials and Methods

Figs. S1 and S2

Table S1

References

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Global Assessment of Organic Contaminants in Farmed Salmon

Ronald A. Hites,^{1*} Jeffery A. Foran,² David O. Carpenter,³ M. Coreen Hamilton,⁴ Barbara A. Knuth,⁵ Steven J. Schwager⁶

The annual global production of farmed salmon has increased by a factor of 40 during the past two decades. Salmon from farms in northern Europe, North America, and Chile are now available widely year-round at relatively low prices. Salmon farms have been criticized for their ecological effects, but the potential human health risks of farmed salmon consumption have not been examined rigorously. Having analyzed over 2 metric tons of farmed and wild salmon from around the world for organochlorine contaminants, we show that concentrations of these contaminants are significantly higher in farmed salmon than in wild. European-raised salmon have significantly greater contaminant loads than those raised in North and South America, indicating the need for further investigation into the sources of contamination. Risk analysis indicates that consumption of farmed Atlantic salmon may pose health risks that detract from the beneficial effects of fish consumption.

Between 1987 and 1999, salmon consumption increased annually at a rate of 14% in the European Union and 23% in the United States (1). Currently, over half the salmon sold globally is farm-raised in Northern Europe, Chile, Canada, and the United States, and the annual global production of farmed salmon (predominantly Atlantic salmon, *Salmo salar*) has risen from ~24,000 to over 1 million metric tons during the past two decades (2). The health benefits of eating fish such as salmon have been well documented (3, 4). However, salmon are relatively fatty carnivorous fish that feed high in the food web, and as such, they bioaccumulate con-

taminants (5). The potential risks of eating contaminated farmed salmon have not been well evaluated. Three previous studies reporting contaminants in salmon are inconclusive because of their very small sample sizes and narrow geographic representation (6–8). As a result, the extent of this problem and the potential risks to human health remain unclear.

We measured organochlorine contaminants in approximately 700 farmed and wild salmon (totaling ~2 metric tons) collected from around the world. We do not report on other important contaminants, such as methylmercury, because our preliminary study (9) showed no significant difference in methylmercury levels between farmed and wild salmon. Using the data on organochlorine contaminants, we assessed the variation in contaminant loads between farmed and wild salmon and among geographic regions, and we calculated the human health risks of salmon consumption. Farmed Atlantic salmon from eight major producing regions in the Northern and Southern hemispheres were purchased from wholesalers that could obtain fish of the appropriate size within the sam-

pling period; in addition, farmed Atlantic salmon fillets were purchased at supermarkets in 16 large cities in North America and Europe. For comparison, samples of five wild species of Pacific salmon [*chum* (*Oncorhynchus keta*), coho (*O. kisutch*), chinook (*O. tshawytscha*), pink (*O. gorbuscha*), and sockeye (*O. nerka*)] were obtained from three different geographic regions. Wild Atlantic salmon were not studied because few are available commercially; nor did we analyze farmed Pacific salmon because they are not raised in any substantial amounts (2, 10).

A total of 594 individual whole salmon were purchased from wholesalers and filleted; an additional 144 fillets were purchased from retailers in Boston, Chicago, Denver, Edinburgh, Frankfurt, London, Los Angeles, New Orleans, New York, Oslo, Paris, San Francisco, Seattle, Toronto, Vancouver, and Washington, DC. Composites of fillets from whole salmon were made on the basis of the location where they were produced (farmed salmon) or purchased (wild salmon). Composites of fillets from retailers were made on the basis of the retail outlet where they were purchased. Each composite sample consisted of fillets from three salmon per location or three fillets per retail outlet, giving 246 measurable samples. All samples were homogenized and analyzed by gas chromatographic high-resolution mass spectrometry (11). Strict quality assurance and quality control procedures were followed (11). Thirteen samples of salmon feed were purchased from the European, North American, and South American outlets of the two major fish feed companies, which together have ~80% of the global market for fish feed (12), and were analyzed as above.

Contaminant concentrations in farmed and wild salmon were compared by analysis of variance. In comparing wild and farmed salmon, farmed salmon were considered as a single group. In addition, locations at which salmon were farmed were compared by analysis of variance with multiple comparisons of means to test for differences among locations in contaminant levels. In all analyses of vari-

¹School of Public and Environmental Affairs, Indiana University, Bloomington, IN 47405, USA. ²Citizens for a Better Environment, Milwaukee, WI 53202, USA. ³Institute for Health and the Environment, University at Albany, Rensselaer, NY 12144, USA. ⁴AXYS Analytical Services, Post Office Box 2219, 2045 Mills Road, Sidney, British Columbia, Canada V8L 3S8. ⁵Department of Natural Resources, ⁶Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY 14853, USA.

*To whom correspondence should be addressed. E-mail: HitesR@Indiana.edu

ance, the replicate composites from each source were not assumed to be independent observations. Differences between farmed and wild salmon and differences among farming locations were consistently substantial and highly significant.

Figure 1 shows the concentrations of 14 organochlorine contaminants in the samples of farmed and wild salmon. Thirteen of these contaminants were significantly more concentrated in the farmed salmon as a group than in the wild salmon [$F = 3.75$, $P = 0.0573$ for lindane; $F = 9.93$, $P = 0.0025$ for hexachlorobenzene (HCB); and $F \geq 11.71$, $P \leq 0.001$ for the other 12 contaminants, with $df = (1, 64)$ for all]. Concentrations in farmed salmon from Europe and from North America were significantly higher than those in wild salmon for all 14 contaminants ($P < 0.05$ for all 28 comparisons). Concentrations in farmed salmon from South America were significantly higher than those in wild salmon for six contaminants [polychlorinated biphenyls (PCBs), dioxins, dieldrin, *cis*-nonachlor, total DDT, and mirex] but significantly lower for two contaminants (HCB and lindane) ($P < 0.05$ for each). In addition, concentra-

tions of all contaminants in farmed salmon from Europe were significantly greater than concentrations in farmed salmon from both North and South America [$F = 8.31$ to 65.87, with $df = (2, 48)$; $P < 0.001$ for all 14 contaminants].

We focused additional analysis on PCBs, dioxins, toxaphene, and dieldrin because the patterns of their occurrence in farmed and wild salmon are similar to the patterns of all contaminants evaluated in this study and because an abundance of human health risk information is available for these compounds (13–19).

The average measured concentrations for these four contaminants are shown in Fig. 2, A to D, as a function of location. As noted above, total PCBs, dioxins, toxaphene, and dieldrin were consistently and significantly more concentrated in the farmed salmon as a group than in the wild salmon [$F = 60.53$, 26.80, 15.03, and 32.22, with $df = (1, 64)$ for all; $P \leq 0.0003$ for all]. Salmon fillets ob-

tained from commercial outlets in the various cities generally clustered with the farmed samples, not with the wild samples.

PCB, dioxin, toxaphene, and dieldrin concentrations were highest in farmed salmon from Scotland and the Faroe Islands and lowest in farmed salmon from Chile and Washington state. Salmon produced in Europe had significantly higher contaminant levels than those produced in both North and South America [$F = 26.15$, 23.36, 64.42, and 59.26, with $df = (2, 48)$ for all; $P < 0.0001$ for all]. Even the least contaminated farmed salmon, from Chile and Washington state, had significantly higher contaminant loads of PCBs, dioxins, and dieldrin than wild salmon [$F = 28.55$, 8.61, and 4.66, with $df = (1, 26)$; $P < 0.0001$, $P = 0.0069$, and $P = 0.0402$, respectively]. Farmed salmon fillets purchased from supermarkets in Frankfurt, Edinburgh, Paris, London, and Oslo were generally the most contaminated, although those purchased in Boston and San Francisco approached these

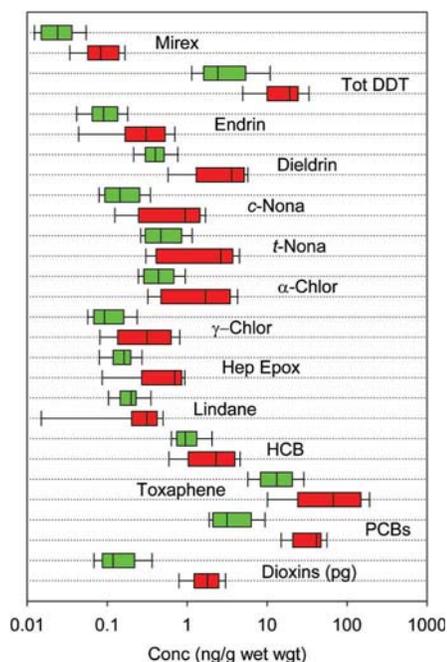


Fig. 1. Concentrations (in ng/g wet weight, except dioxins) of 14 contaminants found in farm-raised (red bars) and wild (green bars) salmon. The vertical lines represent the 10th, 50th, and 90th percentiles, and the boxes represent the 25th to 75th percentiles. Dioxins are in pg of World Health Organization toxic equivalents (WHO-TEQs) per g of wet weight and include polychlorinated dibenzo-*p*-dioxins and dibenzofurans and dioxin-like PCBs. Typically 75% of the total TEQ was due to the dioxin-like PCBs. Other abbreviations are as follows: Tot DDT, the *p,p'* and *o,p'* isomers of DDT, DDD, and DDE; Nona, nonachlor; Chlor, chlordane; Hep Epox, heptachlor epoxide.

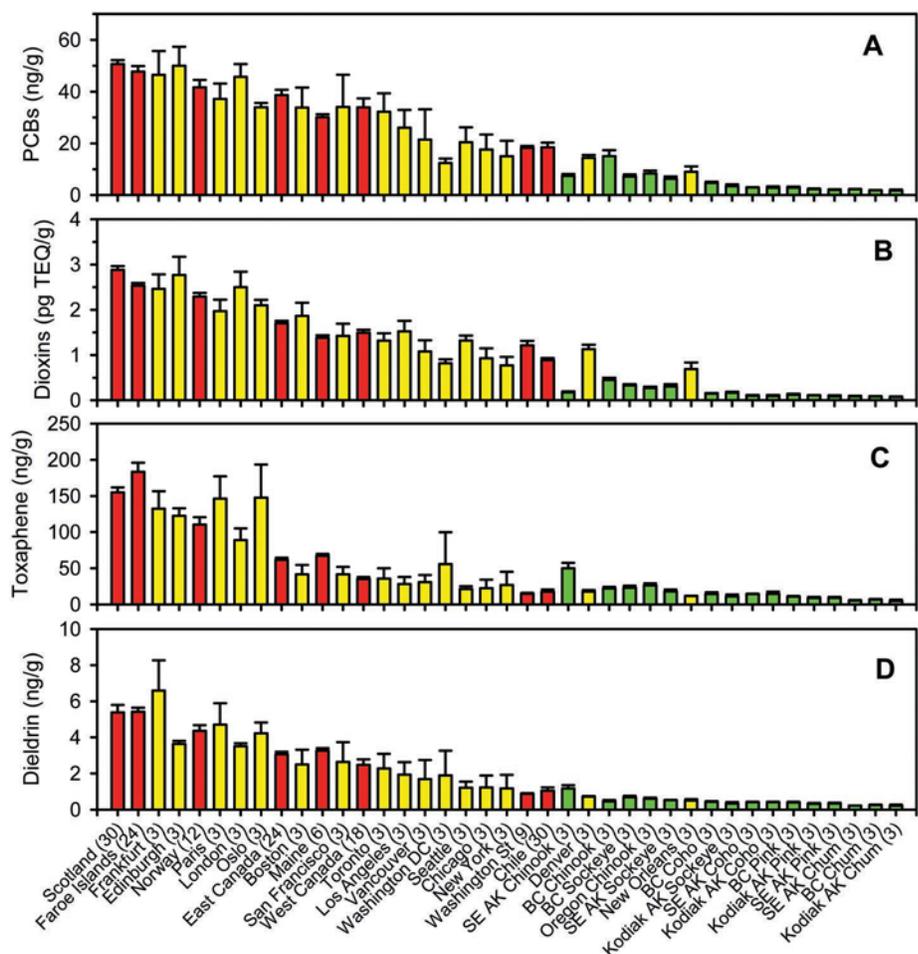


Fig. 2. Concentrations of (A) PCBs in ng/g wet weight, (B) dioxins (for detail, see Fig. 1) in pg of WHO-TEQ/g wet weight, (C) toxaphene in ng/g wet weight, and (D) dieldrin in ng/g wet weight in farmed, supermarket, and wild salmon. The concentrations are all given as functions of the locations where the salmon were grown or purchased. Red represents farmed salmon, green represents wild salmon, and yellow represents salmon purchased at supermarkets. The error bars represent standard errors. The number of samples is given in parentheses after the location identifier. The locations are sequenced by average contaminant rank.

fects and endocrine disruption, occur at lower concentrations than those implicated in cancer (17). However, these hazards were not considered in the present analysis because quantitative risk or threshold levels are not available regarding these effects.

Our data indicate that farmed salmon have significantly higher contaminant burdens than wild salmon and that farmed salmon from Europe are significantly more contaminated than farmed salmon from South and North America. Fish that is not contaminated is a healthy food, high in nutrients, such as omega-3 polyunsaturated fatty acids, that are known to have a variety of beneficial human health effects (3, 4). However, this study suggests that consumption of farmed salmon may result in exposure to a variety of persistent bioaccumulative contaminants with the potential for an elevation in attendant health risks. Although the risk/benefit computation is complicated, consumption of farmed Atlantic salmon may pose risks that detract from the beneficial effects of fish consumption. This study also demonstrates the importance of labeling salmon as farmed and identifying the country of origin. Further studies of contaminant sources, particularly in feeds used for farmed carnivorous species such as salmon, are needed.

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Materials and Methods
Table S1
Reference

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Regulation of Bone Mass in Mice by the Lipoyxygenase Gene *Alox15*

Robert F. Klein,^{1,4*} John Allard,⁵ Zafrira Avnur,⁵ Tania Nikolcheva,⁵ David Rotstein,⁶ Amy S. Carlos,^{1,4} Marie Shea,² Ruth V. Waters,⁵ John K. Belknap,^{3,4} Gary Peltz,⁵ Eric S. Orwoll^{1,4}

The development of osteoporosis involves the interaction of multiple environmental and genetic factors. Through combined genetic and genomic approaches, we identified the lipoyxygenase gene *Alox15* as a negative regulator of peak bone mineral density in mice. Crossbreeding experiments with *Alox15* knockout mice confirmed that 12/15-lipoyxygenase plays a role in skeletal development. Pharmacologic inhibitors of this enzyme improved bone density and strength in two rodent models of osteoporosis. These results suggest that drugs targeting the 12/15-lipoyxygenase pathway merit investigation as a therapy for osteoporosis.

Osteoporosis is one of the most common bone and mineral disorders in all aging communities. It is characterized by low bone mass (and thus, low bone strength), which results in fractures from relatively minor trauma. Although life-style and environmental factors play key roles in the development of osteoporosis, there is now clear evidence that genetic factors are also of great importance (1). Bone mineral density (BMD) achieved in

early adulthood (peak bone mass) is a major predictor of osteoporotic fracture risk. Genetic segregation analyses in inbred mouse strains (2) have identified linkage between peak BMD and several chromosomal regions (or quantitative trait loci, QTLs), but the identities of the underlying genes remain unknown. Recent studies suggest that regulatory variation is important in a variety of complex traits (3). Quantitative gene expression studies can identify genetic variation affecting transcription within genes contributing to differences in complex traits. This is particularly useful for analysis of traits for which a priori gene candidates do not exist.

To identify genes that might regulate BMD, we investigated a region on mouse chromosome 11 that strongly influences peak BMD (4). We generated a DGA/2 (D2) background congenic mouse with an 82-megabase (Mb) region of chromosome 11 replaced by the corresponding region of the C57BL/6 (B6) ge-

¹Bone and Mineral Research Unit, Department of Medicine, ²Department of Orthopaedics and Rehabilitation, and ³Department of Behavioral Neuroscience, School of Medicine, Oregon Health and Science University, 3181 Southwest Sam Jackson Park Road, Portland, OR 97239, USA. ⁴Veterans Affairs Medical Center, 3710 Southwest U.S. Veterans Hospital Road, Portland, OR 97207, USA. ⁵Department of Genetics and Genomics, and ⁶Department of Chemistry, Roche Palo Alto, 3431 Hillview Avenue, Palo Alto, CA 94303, USA.

*To whom correspondence should be addressed: e-mail: kleinro@ohsu.edu