strain SH75 [*MAT***a** *ade6 arg4-17 his4-Sal trp1 tyr7-1 ura3 lys2*::p(GT)$_{14}$G β-GAL] and isogenic deletion strains were used to monitor repeat alterations in the chromosome. To monitor repeat alterations in pSH91, we grew 19 independent 100-μl cultures, each starting from ~10 5-FOA–sensitive cells, in yeast extract–peptone-dextrose (YPD) media for each strain. Cultures were grown to ~10^5 to 10^6 cells before being plated onto medium containing 5-FOA. Rates of tract alteration were determined from the number of 5-FOA–resistant colonies by means of the method of the median (*16*). To monitor tract alterations in pSH31 and in the chromosome, we grew 20 independent 100-μl cultures, each starting from ~10 cells, in YPD for each strain. Cultures were grown to ~10^3 to 10^4 cells before being plated onto medium containing X-Gal. The rate of tract alteration was determined from the number of cultures with no blue colonies (*16*).

19. For each strain, 19 independent 100-μl or 500-μl cultures were grown in YPD. Cells were then plated onto arginine-deficient medium containing canavanine. Rates of forward mutation at the *CAN1*^{s} locus were determined from the number of canavanine-resistant colonies by means of the method of the median (*16*).

20. We thank T. D. Petes and M. Strand for yeast strains, plasmids, and advice and E. Miller and T. Johnson for technical assistance. Supported by National Cancer Institute grant CA 41261 and NIH grant GM 19261.

21 March 1995; accepted 11 May 1995

High Concentrations of Toxaphene in Fishes from a Subarctic Lake

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Concentrations of toxaphene and other organochlorine compounds are high in fishes from subarctic Lake Laberge, Yukon Territory, Canada. Nitrogen isotope analyses of food chains and contaminant analyses of biota, water, and dated lake sediments show that the high concentrations of toxaphene in fishes from Laberge resulted entirely from the biomagnification of atmospheric inputs. A combination of low inputs of toxaphene from the atmosphere and transfer through an exceptionally long food chain has resulted in concentrations of toxaphene in fishes that are considered hazardous to human health.

Lake Laberge (61°11′N, 135°12′W) is located in southern Yukon Territory, Canada. Although the town of Whitehorse has grown along the Yukon River upstream of the lake, Lake Laberge and its watershed are symbols of pristine wilderness for Canadians (*1*). In 1991, a routine survey of contaminants in fishes from Yukon lakes revealed that lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*) from Lake Laberge were contaminated with amounts of toxaphene and other lipophilic contaminants several-fold greater than in the same species from other subarctic and arctic lakes and rivers (*2*, *3*). As a result of the high toxaphene concentrations, fish consumption advisories were issued by Health Canada (*4*), and the commercial, sport, and native subsistence fisheries were closed.

Toxaphene was once used in North America as a piscicide (fish-killing agent) to remove rough fish species and as an agricultural pesticide (*5*). Although Canada and the United States discontinued its use in the early 1980s, it is currently used in Eurasia and Central America and is carried by long-range atmospheric transport to subarctic and arctic regions (*6*). As a result, elevated concentrations of toxaphene have been found in upper-trophic-level biota from the Arctic (*7*), despite their distance from the original source.

One possible cause for the contamination of fishes in Laberge was surreptitious dumping. It is known that one lake in northern central Yukon was treated with toxaphene in 1963 (*8*). Alternatively, the elevated concentrations of toxaphene could be attributed to biomagnification through the food chain because concentrations of persistent chlorinated organics such as toxaphene increase from prey to predator (*9*) and are highest in fish from lakes with the longest food chains (*10*). Significant biomagnification of lipophilic contaminants occurs at subarctic and arctic latitudes, where aquatic organisms must survive winters lasting several months (*11*). Here, we analyzed food-chain organisms, water, and sediments of Yukon lakes for toxaphene to distinguish between the "surreptitious dumping" and "food-chain length" hypotheses.

Traditional studies of contaminant biomagnification in lacustrine food webs have used discrete trophic classifications based on inferred feeding behavior or analyses of stomach contents (*9*, *10*). However, interpretation is complicated for species such as lake trout that may be piscivorous, omnivorous, or insectivorous in different lakes, feeding on varying combinations of terrestrial and benthic insects, other fish, or plankton (*12*). Stable nitrogen isotope ratios ($\delta^{15}N$)

$$\delta^{15}N = [(^{15}N/^{14}N_{sample} \div {}^{15}N/^{14}N_{atmos.\ nitrogen}) - 1] \times 1000$$

have been used as an index of trophic level for freshwater organisms (*13*–*16*). This ratio increases an average of 3 to 5 per mil from prey to predator (*13*, *16*). Stable $\delta^{15}N$ values in fish represent the integral of several months of feeding, thereby providing a continuous measure of trophic position (*17*). Recently, Cabana and Rasmussen (*18*) demonstrated that $\delta^{15}N$ in lake trout muscle is correlated with food-chain length across a large number of lakes. We hypothesized that $\delta^{15}N$ would be directly correlated with contaminant concentrations in individuals varying in trophic position.

Large-volume water samples from six lakes on the Yukon River system and ^{210}Pb-dated sediment core slices from Laberge, Fox, and Kusawa lakes (*19*) were used as surrogate measures for toxaphene inputs

Table 1. Surface and maximum fluxes of toxaphene (in nanograms per square meter per year) and the median dates of slices in sediment cores from lakes in Yukon Territory [YT; this study (*21*)], Northwest Territories [NWT; (*26*)], and two lakes treated with toxaphene in Alberta [AB; (*25*)], Canada. Peanut and Chatwin fluxes were not corrected for sediment focusing, and the mean dates of these slices are given.

Lake	Surface flux	Date	Maximum flux	Date
Laberge-2, YT (61°11′N, 135°12′W)	180	1991	270	1978
Laberge-3, YT	32	1991	340	1973
Fox, YT (61°14′N, 135°28′W)	180	1992	260	1983
Kusawa, YT (60°20′N, 136°22′W)	24	1989	140	1974
Far, NWT (63°42′N, 90°40′W)	310	1986		
Hawk, NWT (63°38′N, 90°42′W)	500	1985		
Amituk, NWT (75°03′N, 93°48′W)	140	1980		
Hazen, NWT (81°45′N, 71°30′W)	120	1986		
Peanut, AB (54°01′N, 114°21′W)	2.3×10^4	1985	1.0×10^5	1962
Chatwin, AB (54°15′N, 110°51′W)	1.7×10^4	1990	5.1×10^5	1963

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from atmospheric or point sources. The total toxaphene concentration in Laberge water (23 pg liter^{-1}) was within the range found for five other lakes in southern Yukon [12 to 27 pg liter^{-1} (*20*)]. Surface and maximum fluxes of toxaphene in the sediment cores were comparable in all three lakes, similar to those found in other arctic cores and orders of magnitude lower than values found in temperate lakes previously treated with toxaphene (Table 1) (*21*). We thus dismissed the hypothesis that Laberge received elevated inputs of toxaphene from point sources.

Concentrations of toxaphene in biota were correlated with their trophic position, as established by $\delta^{15}N$ (Fig. 1). Further, the slopes of the toxaphene concentration versus $\delta^{15}N$ in the three lakes were not significantly different, indicating a broad regional similarity in the biomagnification of this contaminant through the food chain. The least square regression equations of the logarithm of toxaphene (in nanograms per gram of wet weight) versus $\delta^{15}N$ (per mil) ($\pm$ the estimated SE) (*22*) are as follows:

$$\text{Laberge: } \Sigma\text{tox.} = 0.23\ (\pm\ 0.03)\delta^{15}N - 0.33\ (\pm\ 0.23),\ SE_{est} = 0.56$$

$$\text{Fox: } \Sigma\text{tox.} = 0.19\ (\pm\ 0.08)\delta^{15}N - 0.70\ (\pm\ 0.63),\ SE_{est} = 0.60$$

$$\text{Kusawa: } \Sigma\text{tox.} = 0.25\ (\pm\ 0.04)\delta^{15}N - 0.59\ (\pm\ 0.31),\ SE_{est} = 0.39$$

However, values of $\delta^{15}N$ for lake trout, burbot, and lake whitefish (*Coregonus clupeaformis*) from Laberge were significantly higher than values for the same species from other lakes, indicating that these species feed at a higher trophic level (Fig. 2). This observation is consistent with summer stomach analyses of fishes from the three lakes, which revealed that Laberge lake trout, burbot, and occasionally lake whitefish are piscivorous, whereas the same species in other nearby lakes ate largely invertebrates, with fish as a minor component of their diets (*15*, *23*). Because of their higher trophic position, fishes from Laberge accumulated more toxaphene than the same species from surrounding lakes (see Fig. 1).

Taken together, the contaminant-$\delta^{15}N$ relations for biota and contaminant concentrations in water and sediments indicate that a longer than normal food chain is the sole reason for the elevated toxaphene concentrations in fishes from Lake Laberge. We speculate that this difference in food-chain length may be due to sustained heavy fishing pressure on Lake Laberge. Fish form a critical food base for many populations of aboriginal North Americans (*7*, *24*). Screening the fisheries of northern lakes for organic contaminants would be an expensive and time-consuming undertaking. Our results suggest that the highest contaminant concentrations are found in fishes from lakes with exceptionally long food chains and that $\delta^{15}N$ might be useful as an initial screening mechanism to identify such lakes.

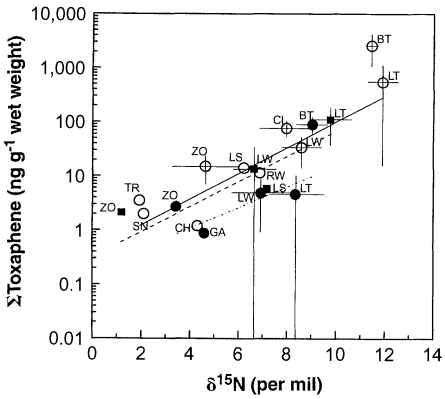

Fig. 1. Mean ($\pm$ SD, n = 1 to 14) of $\delta^{15}N$ versus Σtoxaphene for whole invertebrates and fish muscle (burbot liver was used for toxaphene analyses) from Laberge (○, solid line), Fox (●, dash-dot line), and Kusawa (■, dashed line) lakes, Yukon Territory. Organisms are identified as follows: LT, lake trout; BT, burbot; LW, lake whitefish; RW, round whitefish; CI, least cisco; LS, longnose sucker; ZO, zooplankton; GA, *Gammarus* sp.; CH, chironomid (subfamilies Tanypodinae, Prodiamesinae, and Chironominae); SN, snail (family Lymnaeidae); and TR, tricopteran (family Limnephilidae) (*27*).

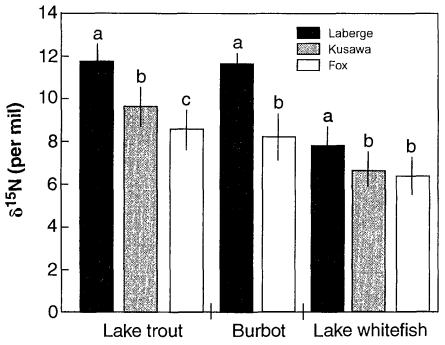

Fig. 2. Mean ($\pm$ SD, n = 5 to 14) $\delta^{15}N$ for lake trout, burbot, and lake whitefish muscle from Laberge, Fox, and Kusawa lakes, Yukon Territory, Canada (*28*).

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19. Fox and Kusawa lakes were chosen as reference locations because of their proximity to Lake Laberge and similar fish species.
20. Epilimnetic water samples (89 to 95 liters) were collected from Atlin (60°00′N, 133°50′W), Tagish (60°10′N, 134°20′W), Marsh (60°25′N, 134°18′W), Laberge, Fox, and Kusawa lakes in February 1994. We extracted unfiltered water samples using a Goulden continuous extractor [G. D. Foster and P. F. Rogerson, *Int. J. Environ. Anal. Chem.* **41**, 105 (1990); P. Goulden and D. J. H. Anthony, *NWRI Contribution 85-121* (Analytical Methods Division, National Water Research Institute, Burlington, Ontario, 1985)]. We analyzed both water and sediment extracts using high-resolution electron-capture negative-ion mass spectroscopy, following the conditions given in (*25*). The detection limit for total toxaphene was <50 pg μl^{-1} with a resolving power of 15,000.
21. We collected sediment cores from Laberge, Fox, and Kusawa lakes in March 1992 and March 1993 using a KB corer. Cores were sliced at 1-cm increments and kept at 0° to −10°C both before and after they were freeze-dried. We extracted sediment slices using the methods given in (*26*). Excess ^{210}Pb profiles were log-linear and were used to calculate the median age of each slice. Integrals of excess ^{210}Pb ranged between 0.189 and 0.259 becquerel per square centimeter for all three lakes, indicating little among-lake difference in sediment focusing. We calculated focusing factors using latitude-specific atmospheric fluxes of ^{210}Pb. We calculated sedimentation rates (in grams per square meter per year) using both a simple linear model (assumes constant ^{210}Pb flux and constant dry-mass sedimentation rate) and a Robbins rapid steady-state mixing model [assumes constant ^{210}Pb flux and accumulation rate and a rapid steady-state mixing only throughout a surface zone of fixed thickness; J. A. Robbins, in *Biogeochemistry of Lead in the Environment*, J. O. Nriagu, Ed. (Elsevier, Amsterdam, 1978), pp. 285–293]. The Robbins model values were used to calculate fluxes of toxaphene; however, for both models comparable sedimentation rates were calculated (within ±10%). Fluxes were calculated as toxaphene concentration (nanograms per gram of dry weight) multiplied by the sedimentation rate divided by the focusing factor.
22. Laberge: $r^2 = 0.62$, $n = 43$, $P < 0.01$; Fox: $r^2 = 0.25$, $n = 18$, $P = 0.03$; Kusawa: $r^2 = 0.69$, $n = 23$,

$P < 0.01$; r, correlation coefficient.
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27. We obtained fish and invertebrates from these three lakes by netting or dredging in June to August of 1992 and 1993. Samples were frozen shortly after collection and kept at −40°C until analyzed for toxaphene and $\delta^{15}N$ according to the methods in (*15*). There were no significant differences between the slopes of the regressions with the use of analysis of covariance ($P < 0.05$). Similar relations between $\delta^{15}N$ and other persistent chlorinated organics were also found (*15*).
28. Tukey multiple comparison (lake trout and lake whitefish) and Student's t tests (burbot) were used to test for significant differences. Within species, bars with the same letter were not significantly different ($P < 0.05$).
29. Research was supported by the Arctic Environmental Strategy Northern Contaminants Program (Indian and Northern Affairs, Canada), the Arctic Institute of North America Fellowship (Alberta), a Canadian Circumpolar Institute Boreal Alberta research grant (Alberta), the Northern Research Institute Fellowship (Yukon), a Northern Scientific Training Program grant (Indian and Northern Affairs, Canada), a Natural Science and Engineering Research Council (Canada) operating grant to D.W.S., and a Tri-Council Eco-Research doctoral fellowship to K.A.K. We thank B. Billeck, L. Chin, B. Danell, D. Davidge, N. deGraff, J. Eamer, T. Genier, S. Kidd, S. Lamontagne, B. Lendrum, S. Lendrum, D. Metner, A. Omelchenko, M. Palmer, B. Parker, G. Pope, S. Thompson, A. von Finster, P. Wilkinson, the Department of Fisheries and Oceans (Yukon), the Department of Renewable Resources (Yukon Government), Environment Canada (Yukon), Ta'an Kwach'an First Nation, and the Yukon Contaminants Committee for assistance. We thank M. Alaee (Environment Canada, Burlington, Ontario) for providing water extracts; R. Currie, B. Grift, K. Koczanski, S. Mohammed, P. Ramlal, B. Rosenberg, and G. Stern for laboratory analyses; and B. Miskimmin for reviewing an earlier draft of the manuscript.

3 February 1995; accepted 26 May 1995

A Role in B Cell Activation for CD22 and the Protein Tyrosine Phosphatase SHP

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CD22 is a membrane immunoglobulin (mIg)–associated protein of B cells. CD22 is tyrosine-phosphorylated when mIg is ligated. Tyrosine-phosphorylated CD22 binds and activates SHP, a protein tyrosine phosphatase known to negatively regulate signaling through mIg. Ligation of CD22 to prevent its coaggregation with mIg lowers the threshold at which mIg activates the B cell by a factor of 100. In secondary lymphoid organs, CD22 may be sequestered away from mIg through interactions with counterreceptors on T cells. Thus, CD22 is a molecular switch for SHP that may bias mIg signaling to anatomic sites rich in T cells.

The diverse array of antigen receptors on B and T lymphocytes, (mIgs and T cell receptors, respectively) can bind an almost unlimited number of different antigens. Recombination among the genetic elements that encode these receptors accounts for this diversity, but it does so without reference to potential antigens and creates receptors that are potentially self-reactive. The solution to this problem, as first suggested by Bretscher and Cohn (*1*), is to require a second signal for the clonal expansion and differentiation of antigen-specific B cells into plasma cells. This second signal is provided by helper T cells in the form of gp39 (*2*), the ligand for CD40, during its cognate interaction with the antigen-reactive B cell. In the absence of a second signal, the first signal of mIg induces tolerance or death of the B cell (*3*). There is a potential difficulty with this strategy: the low frequency of primed, antigen-specific helper T cells. This problem may be resolved by restriction of the activation of B cells by antigens to secondary lymphoid organs. T cells recirculate through these structures in large numbers, thus increasing the potential for encounters between antigen-specific B and T cells. To date, no mechanism has been described that could provide this level of control.

CD22 is a membrane protein (*4*) that may enable the B cell to sense the presence of adjacent lymphocytes and regulate signaling by mIg. The NH_2-terminal Ig-like domains of the extracellular region of CD22 (*5*) have specificity for glycoconjugates containing α 2,6–linked sialic acid that are expressed preferentially by B and T cells (*6*). CD22 associates with mIg, and its intracellular domain is tyrosine-phosphorylated after ligation of mIg (*7*), enabling interaction with phosphotyrosine-specific SH2 domains of intracellular signaling proteins.

The protein tyrosine phosphatase (PTP) SHP (also termed PTP1-C, SHPTP1, and HCP) contains two SH2 domains (*8*). The *SHP* gene is mutated in *motheaten* (*me*) and *viable motheaten* (me^v) mice (*9*) that have an expanded B-1 subset of B cells, elevated concentrations of immunoglobulin M (IgM) and IgG3 (*10*), and autoimmune arthritis and glomerulonephritis. Recently, me^v B cells were shown to induce the release of intracellular calcium in response to lower concentrations of antigen than did normal B cells (*11*).

We determined whether tyrosine-phosphorylated CD22 interacts with SHP by precipitating the PTP from NP-40 lysates of Daudi B lymphoblastoid cells that were resting or had been activated by ligating mIgM (*12*, *13*). The proteins were resolved by SDS–polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose, and immunoblotted sequentially with antibody to phosphotyrosine and antibody to SHP. In resting Daudi cells, SHP was not tyrosine-phosphorylated, and it coimmunoprecipitated a phosphorylated protein of approximately 130 kD that was barely detectable (Fig. 1A). In activated cells, the PTP was tyrosine-phosphorylated and was associated with phosphorylated proteins of 120 to 130 kD and 72 kD. We immunoprecipitated CD22 from replicate samples of the Daudi cells and subjected the precipitates to the same immunoblot analysis. The tyrosine-phosphorylated triplet of CD22 comigrated with the SHP-associated triplet at 120 to 130 kD. The increase in phosphorylation of CD22 induced by mIgM was associated with an increase in the amount of coimmunoprecipitating SHP (Fig. 1B). The SHP that was associated with CD22 appeared to be relatively less phosphorylated than the total SHP, which suggests that it was the tyrosine phosphorylation of CD22, rather than of SHP, that led to the binding of the PTP.

To determine which of the six tyrosines of the cytoplasmic domain of CD22 mediate the interaction with SHP, murine splenic B cells were permeabilized and incubated with buffer alone or buffer containing each of six phosphotyrosyl peptides corresponding to the sequence of

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