Risk of POP mixtures on the Arctic food chain

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Abstract

The exposure of the Arctic ecosystem to persistent organic pollutants (POPs) was assessed through a review of literature data. Concentrations of 19 chemicals or congeneric groups were estimated for the highest levels of the Arctic food chain (arctic cod, ringed seals, and polar bears). The ecotoxicological risk for seals, bears and bear cubs was estimated by applying the concentration addition (CA) concept. The risk of POP mixtures was very low in seals. By contrast, for adult polar bears the risk was two orders of magnitude higher than the risk threshold and even more (three orders of magnitude above the threshold) for bear cubs fed with contaminated milk Based on the temporal trends available for many of the chemicals, the temporal trend of the mixture risk for bear cubs was calculated. Relative to the 1980s, a decrease of the risk from the POP mixture is evident, mainly due to international control measures. However, the composition of the mixture substantially changes and the contribution of new POPs (particularly perfluorooctane sulfonate, PFOS) increases. These results support the effectiveness of control measures, such as the Stockholm Convention, as well as the urgent need for their implementation for new and emerging POPs.

Key words: POPs, mixture, risk assessment, Arctic, penalized regression smoothers

Introduction

Persistent organic pollutants (POPs) are chemicals that remain intact in the environment for long periods, travel long distances, accumulate in living organisms, and are toxic to humans and wildlife. POPs are capable of transport via air, water, migratory species, and technical matrices such as products and wastes. Therefore, they become ubiquitous in the environment. Their potential for long-range transport is the primary reason for regional, continental, and global distribution [1, 2]. The different physical and chemical properties of these substances (differences in persistence, water solubility, mechanisms of bioaccumulation, mechanisms of toxic effects) also present a number of challenges [3, 4].

Control and management of POPs is hindered by their complex emission patterns and releases into the environment. Some POPs were primarily applied in agriculture, meaning that they were directly released into the environment where they contaminated abiotic compartments and living organisms. Some were produced and applied as industrial products or intermediates. Many are produced unintentionally as by-products of various industrial and combustion processes and by the natural transformation of primarily released substances.

The Stockholm Convention on POPs [5] is a global treaty focused on substances, which are toxic, resistant to degradation, and have strong potential to accumulate in humans and other living organisms. In the first formulation, the Convention addressed a series of legacy POPs known as the “dirty dozen” (like polychlorinated biphenyls – PCBs and dioxins). Subsequently, the list has been periodically updated with the inclusion of new chemicals, including legacy and emerging POPs.

The strict regulation and, in many cases, the total ban of the most harmful POPs will reduce the global impact of these chemicals. However, due to their persistence and biomagnification potential, the presence of POPs in the global environment is likely to represent a risk to wildlife and humans for decades.

At present, this risk mainly depends upon a wide spectrum of sources for these substances, including former production and application processes as well as new types of environmental emissions. Environmental compartments, which have been highly contaminated for decades, serve as important sources of the current degree of contamination. These secondary sources, developed mainly during the second half of the last century, represent a significant fraction of the globally distributed expanse of chemicals and are an important source of contamination, especially in remote areas.

Wildlife and humans from polar areas are subject to a level of risk that may be substantially higher in comparison to tropical and temperate regions where major emissions took place. This is due to the long-range distribution patterns of POPs and their persistence in cold climates, as well as the diet and the high fat content of organisms at higher trophic levels. Since the late 1960s, the presence of detectable concentrations of POPs and the relevant risk for the biological community have been discovered and documented in Antarctica and in the Arctic [6-11].

Due to distribution and fate patterns, the Arctic environment is exposed to a complex mixture of POPs that exhibits a composition much different from those typical of emission areas and that is subject to changes over time, with a progressive decrease of legacy POPs and a possible increase of emerging contaminants. Extensive programmes have been developed for studying POP contamination in the Arctic [12-16] and several reviews described the temporal trends in biotic and abiotic matrices of these compounds before and after the Stockholm Convention [see, for example, 10, 16-21].

The effects of POPs on the Arctic ecosystem have also been studied, particularly considering biomagnification and consequences on the organisms on the top of the food chain (such as seals and polar bear) [see, for example, 17, 22-25]. In spite of the large bulk of information on these topics, a characterisation of the risk of the mixture of POPs for a simplified Arctic food chain (cod, seal, polar bear), comparing environmental concentrations with a given toxicological endpoint, has never been attempted. The purpose of this study, based on an extensive review of monitoring data produced over the last four decades, was to estimate the composition of the POP mixture likely present in recent years within the Arctic environment and to assess the risk of the mixture for the Arctic animals on the top of the food chain (ringed seal, polar bear). Trends over time for the mixture composition and risk were also estimated, highlighting the changing ecotoxicological role of individual components. The work also contributes information that could be used to assess the effectiveness of control measures (in particular, the Stockholm Convention) in reducing the global risk of POPs, to estimate the time needed for a substantial reduction of the risk of legacy POPs, and to highlight future research priorities on emerging potential POPs.

Materials and methods

The selected chemicalsAll of the original POPs, most new POPs and POPs candidates listed in the most recent iterations of the Stockholm Convention were considered. (see Supplementary Material, Table S-1). Some chemicals were excluded (e.g. chlordecone, pentachlorobenzene) due to the lack of detailed information on the concentrations in the various matrices of the Arctic ecosystem.

The list of the compounds considered (see Table 1) includes some individual chemicals, often present in the environment together with some analogous compounds (congeners, isomers, metabolites) and some large groups of congeners or similar compounds. The complete description of the selected chemicals is reported in the Supplementary Material (Section 2).

Study area and food chain

The data collected cover a large sector of Arctic and sub-Arctic regions. Most data refer to the area between Svalbard Islands and Alaska. Only the Russian Arctic is poorly covered. The distribution of sampling areas and the quantitative coverage of different Arctic regions is shown in figure S-1 of the Supplementary Material.

In order to describe the behaviour of POP mixtures in the Arctic ecosystem, a simple food chain has been considered. Data on POP concentrations have been collected for fish (Arctic cod, *Boreogadus saida*), ringed seal (*Pusa hispida*) and polar bear (*Ursus maritimus*) as one of the most representative Arctic top predators, classified by the International Union for Conservation of Nature [26] as “Vulnerable". The Arctic cod-ringed seal-polar bear food chain is a very well defined food chain, typical for a low biodiversity ecosystem like the Arctic, with simple predator-prey relationships [27].

The diet composition of polar bears has already been studied extensively. Polar bears predominately eat the blubber and meat of ringed seals (*Phoca hispida*) or other seal species, as well as other marine mammals [28]. The effects of climate changes may affect the diet of polar bears [29] and diet composition may affect the contaminant burden. However, the consequences of global changes have not been considered in this work and the main assumption was that polar bear fed only on ringed seal. A similar diet simplification was assumed recently by Pavlova *et al*. [30] in their modelling exercise.

Sources of variability and uncertainty in the risk assessment

The fundamental hypothesis assumed is that in remote areas, far from emission sites, where only long range transport may occur, environmental and geographical characteristics are the main factors affecting the environmental concentrations of POPs. It may be supposed that, due to many characteristics (relatively uniform cold temperature, absence of dry land, etc.), the Arctic region, as defined by AMAP (Arctic Monitoring and Assessment Programme) [12], is a relatively consistent area, at least for the purposes of a general assessment like those proposed in this paper. Therefore, POP concentrations may be assumed as relatively homogeneous and data from different literature sources may be considered as comparable.

These assumptions must be taken with some care and referred to the objective and the scale of this work. Meteorology and transport of POPs via air, ocean currents and rivers is different between Arctic sub-regions. This may produce differences in water concentrations in the different Arctic basins.

Moreover, in top predators like polar bears, concentrations may be affected even by regional dietary differences [31]. Additional sources of variability derive from the origin of literature data (different survey programs, different sampling procedures, different laboratories, etc.) as well as other confounding factors such as sex and age.

Mc Kinney *et al.* [31] showed that in 11 polar bear populations, distributed from Svalbard to Alaska, the variability of concentrations of PCBs and PBDEs is within a factor of about 3, excluding only the two very southern populations of the Hudson Bay located outside of the Arctic Circle. Even including these populations, the variability is within one order of magnitude. In ringed seals and polar bears from the North American Arctic (Canada and Alaska) Braune *et al*. [18] observed a moderate variability, usually within a factor of 3, for many legacy POPs. They observed that the effects of sex and age may be more relevant that geographic differences.

We are aware that this variability is not negligible. However, the objective of this work is not a precise description of POP distribution into the Arctic. Indeed, it is evaluating the ecotoxicological risk determined by POP concentrations that might realistically occur in the Arctic environment. For a large-scale assessment (in space and time) of ecotoxicological risk from POP mixture, like those performed in this work, a certain level of variability may be assumed as affecting only marginally the general assessment and conclusions. Considering the sources of uncertainty in this kind of assessment (toxicity data, application factors, mixture assessment, etc.), a geographic variability within a factor of 2 or 3 may be assumed as acceptable.

The entire data collected for this work confirms the hypothesis of a moderate spatial variability. The variability of concentrations of the same chemical in samples collected in different Arctic sampling areas over sampling periods of five years is relatively small, usually not higher than a factor of 3. This confirms the hypothesis that in the Arctic the variability of POP pollution, determined by long-range transport, is not comparable with those observable in emission sites. This also confirms the suitability of the collected data for the objectives of this work. Details of the assessment of the variability within the data set are reported in the Supplementary Material (Section 4, Table S-3).

In order to perform a risk assessment of POP mixture, the realistic quantitative composition of the mixture that includes all the 19 chemicals (or congeneric groups) selected has to be defined. . The number of analysed POPs varies substantially by publication. Therefore, finding data on all the 19 selected chemicals analysed in the same sample was impossible. Thus, different papers reporting data on different POPs in a given environmental matrix, referred to a given temporal window, were used for the assessment of the composition of the mixture. This result may be assumed as a “realistic” mixture for the Arctic environment, even if data do not refer strictly to the same geographic area. This procedure is also supported by the moderate spatial variability as described above.

Exposure data

Literature survey. The published data on the POP concentrations in Arctic biota, from scientific journals or technical reports available online, were collected.

The sampling period considered for data selection included more than 40 years (from the late 1960s to 2011). For assessing the risk to biota, the average of a five-year time period (2006-2010) was used. For this time period, data were available for most of the chemicals, at least for seals and polar bears. If not available, data from the previous five year period (2001-2005) were used. More recent data are still rare in the literature.

For lipophilic chemicals, the concentrations in the whole body of *Boreogadus saida* and in fat of *Pusa hispida* and of *Ursus maritimus* were considered for this study. The results were normalised to lipid content; data given in the original paper on a dry or wet weight basis were converted on a wet weight lipid basis. A different approach was used for perfluorinated compounds (PFOA and PFOS) that are water-soluble and accumulate in proteins instead of lipids. For these chemicals, data were expressed as wet weight concentration in whole body for fish and in liver for seal and bear.

Age and sex of the analysed organisms were not considered as a discriminating factor. Particularly in older papers, these details were not reported. Moreover, additional reasons for supporting this choice are better described in the discussion section.

If a given paper reported multiple observations for a single chemical, the geometric mean of all values was calculated. In some papers, the geometric mean was directly reported by the authors. All the geometric means of single papers, referred to the selected temporal window (2006-2010), were collected to calculate the final geometric mean for the period, weighted as a function of the number of observations. The obtained concentration values, reported in Table 1, may be considered as the realistic concentrations in biota in the Arctic for the selected temporal window.

Calculated data. Some missing data have been estimated through calculation. In particular, concentrations of lipophilic chemicals in polar bear milk have been calculated from concentrations in bear fat, assuming equilibrium between body and milk lipids and a lipid concentration in milk of 33% [32]:

$$C_{milk}\ (\mathrm{ng/g\ ww}) = C_{bear}\ (\mathrm{ng/g\ lw}) * 0.33 \qquad [1]$$

For perfluorinated chemicals, which accumulated on proteins, concentrations in milk have been calculated from concentrations in bear liver assuming an equilibrium between liver and milk proteins and a protein concentration in milk and in liver of 10% and 20%, respectively [32, 33]:

$$C_{milk}\ (\mathrm{ng/g\ ww}) = C_{liver}\ (\mathrm{ng/g\ ww}) * 0.5 \qquad [2]$$

Toxicity data

The most appropriate end-point, suitable for assessing the risk from such a complex mixture should be selected, particularly taking into account that it is a multicomponent mixture with individual components present at low or very low concentrations or doses. The different classes of POPs may have completely different toxicological modes of action. Many of them are known to be endocrine disruptors. Many other effects are known, at least for humans and mammals (neurotoxicity, immunotoxicity, liver toxicity, etc.).

However, precise end-points, that in human toxicology are often referred to a specific target organ, are meaningless in ecotoxicology due to the different objective of environmental protection (protecting structure and functions of the biological community) and of human health protection (protecting individuals). Moreover, in ecotoxicology, knowledge on the toxicological modes of action on all the different types of organisms that may be present in an ecosystem is largely incomplete. Finally, in spite of the recognised vulnerability and fragility of the polar ecosystem, very few specific data are available on toxic effects of contaminants to polar biota [22, 23].

To describe the toxicological behaviour of a mixture of chemical substances, two different approaches may be used: the Concentration Addition (CA) or the Independent Action (IA) model. The two models are applicable to chemicals with the same mode of action or with different mode of action respectively. However, our current status of knowledge may justify the general use of CA as a pragmatic default approach to the predictive hazard assessment of chemical mixtures [34]. The use of CA as a reasonable worst-case approach to the predictive hazard assessment of chemical mixtures has been supported in a recent document of the European Commission [35]. A more detailed justification of the use of the CA approach is reported in the Supplementary Material (Section 5).

Therefore, in this paper, the risk of mixtures has been estimated using CA as a worst-case approach. Risk has been calculated at three different levels:

- seals eating fish;
- adult bears eating seals;
- bear cubs consuming milk.

The risk to fish was not calculated due to the lack of comparable long-term toxicity data for all the chemicals considered.

For all types of effects, in order to apply the CA approach, the same toxicological endpoint must be used for all chemicals considered. Moreover, due to the problem of long-term exposure to relatively low doses, long-term toxicity data should be preferred. Finally, most POPs being endocrine disrupting chemicals, end-points dealing with reproduction and development should also be preferred.

Those listed above are the optimal requirements. However, one must be aware that the most relevant drawback in selecting a suitable end-point is the availability of reliable toxicity data for the same end-point for all the components of the mixture. Therefore, the only possibility to perform at least a preliminary assessment is accepting rough compromises, using those data that are available and relatively homogeneous for all the chemicals examined.

For mammals, several types of short and long-term data were available. However, the comparability of methods and end-points was quite difficult. In absence of suitable data on the same relevant end-point for all chemicals, it was decided to use the Hazard Index (HI) approach [36, 37]. Hazard Quotients (HQs) for individual chemicals were calculated using as Reference Value (RV), the ADI (Acceptable Daily Intake) proposed for protecting human heath by international organisations (WHO, FAO, EFSA, US EPA).

$$\mathrm{HI}_{\mathrm{mix}} = \sum_{i=1}^{n} \mathrm{HQ}_{\mathrm{i}} = \sum_{i=1}^{n} \frac{\mathrm{TDI}_{\mathrm{i}}}{\mathrm{ADI}_{\mathrm{i}}} \qquad [3]$$

where HI_{mix} is the hazard index of the mixture; HQ_i is the hazard quotient of the individual chemical; TDI_i is the total daily intake of the individual chemical and ADI_i is the acceptable daily intake of the individual chemical.

The following HQs have been considered:

- seal HQs calculated as the ratio between TDI (fish eating) and mammals ADI;
- bear HQs calculated as the ratio between TDI (seal eating) and mammals ADI;
- bear cub HQs calculated as the ratio between TDI (milk eating) and mammals ADI.

TDI is calculated according to the following equation:

$$TDI = DFI * C_F \qquad [4]$$

where DFI is the daily food intake (fish, seal fat and milk for seals, bears and bear cubs, respectively) and C_F is the concentration in food.

For TDI calculations, following assumption have been made:

1. TDI for seals:
   - daily food intake= 7% of body weight per day = 0.07 kg ww/kg bw [38]
   - lipid content of fish: 7% = 0.07 kg/kg bw [39].

$$TDI_{seal}\ (mg/kg\ bw) = (0.07 * C_{fish} * 0.07)/1000 \qquad [5]$$

   where, C_{fish} is the concentration of the chemical in fish (μg/kg lw)

2. TDI for bears:
   - polar bears eat mainly seal fat [40], in this assessment it was assumed that the whole food requirement is covered by fat;
   - daily food (seal fat) intake= 2% of body weight per day = 0.02 kg ww/kg bw [40]

$$TDI_{bear}\ (mg/kg\ bw) = (0.02 * C_{seal})/1000 \qquad [6]$$

   where, C_{seal} is the concentration of the chemical in seals (μg/kg lw)

3. TDI for bear cubs:
   - daily food (milk) intake= 20% of body weight per day = 0.2 kg ww/kg bw [40]

$$TDI_{bear\ cubs}\ (mg/kg\ bw) = (0.2 * C_{milk})/1000 \qquad [7]$$

   where, C_{milk} is the concentration of the chemical in bear milk (μg/kg ww)

We are aware that the described approach presents some critical issues. In particular:

- The ADI developed for humans is used as a reference endpoint for Arctic mammals toxicity. We assumed that it was a possibility for using a comparable reference endpoint for all chemicals considered. Toxicity data of different POPs on seals and bears are rare and hardly comparable. Therefore, extrapolating a protective value developed for a mammal species (man) to others mammalian species (seal and bear) was assumed as the best solution for getting a reasonable indicative value for a protective endpoint.

- The objective of human risk assessment (protecting individuals) is different from the objective of ecotoxicological risk assessment (protecting structure and functions of ecosystems). However, for a threatened species, as polar bear is, the protection of individuals may be relevant also on an environmental point of view. Moreover, seals and bears are K strategic species and are keystone species for the Arctic ecosystem. Therefore, changes in their populations may produce substantial alterations in structure and functioning of the system.

Statistical analysis

For those chemicals with enough data available for a long temporal window, a time trend has been described. Linear regression is clearly unsuitable to model temporal trends of log-concentrations of contaminants due to nonlinearity in the true pattern. Moreover, quantifying the ecological risk that such contaminants will exceed reference levels is a fundamental issue. Therefore, a suitable statistical method reliable from the predictive perspective is needed. Non parametric regression [41] has been recently used to model environmental time series (see for example [42, 43]). In particular, the conditional expectation of the response variable Y_i (contaminant log-concentration) is assumed to be equal to a smooth function $g(x_i)$ of the covariate x_i (i.e. time), $i = 1,..,n$. A widespread approach to estimation of such models is based on loess [44, 45], which is a local polynomial regression with variable bandwidth based on nearest neighbor.

Here, we prefer to focus on penalized regression smoothers based on splines [46, 47], because of their appealing theoretical properties. More precisely, the function g is represented as a linear combination of completely known basis functions, so that only the coefficients of the combination need to be estimated (typically by minimizing a least squares fitting objective). The cubic spline basis is particularly well suited as it can be shown to have good approximation theoretic properties. Such spline is a curve made up of sections of cubic polynomials, joined together, so that they are continuous in value as well as first and second derivatives. The points at which the sections join are known as the knots and, typically, they are chosen in an evenly spaced way through the range of the observed covariate. The degree of smoothing is controlled by adding a roughness penalty to the objective function, so that a modified least squares criterion (see [47]) should be minimized, i.e.

$$\sum_{i=1}^{n}[y_i - g(x_i)]^2 - \gamma \int [g''(x)]^2 dx \qquad [8]$$

where γ is the smoothing parameter. This represents the extent to which roughness is penalized and, therefore, it allows to control the trade-off between model fit and model smoothness, providing flexibility in the presence of fast or slow changing trends. Notice that the cubic spline arises naturally from the specification of the above least squares criterion, as it can be shown to minimize it among all functions that are continuous on the range of the covariate and have absolutely continuous first derivatives.

The fundamental choice of the smoothing parameter value can be accomplished via cross validation (CV), which minimizes an estimate of the mean squared error in predicting a new variable. In particular, we prefer to adopt generalized CV instead of ordinary one because of computational gains as well as invariance properties. This allows choosing the value of the smoothing parameter which represents the best one from a predictive perspective.

Computing confidence intervals (both for the model parameters and for the smooth terms) as well as performing hypothesis testing need a quantification of the uncertainty of the estimators.

This can be accomplished on the basis of frequentist approach to inference, i.e. the classical approach based on repeated sampling principle, as the estimators can be shown to be asymptotically unbiased and normally distributed. Unfortunately, it is well known that, when smoothing parameters have been estimated, the p-values are typically lower than they should be, meaning that the test rejects the null hypothesis too readily.

That is why we preferred to use a Bayesian approach which results in a posterior distribution for the parameter estimators and for quantities derived from them (note that, for non-normal data, posterior normality of the estimators is again an approximation justified by large sample results). Therefore, p-values for smooth terms have been based on a test statistic motivated by an analysis of frequentist properties of Bayesian confidence intervals [48], which show better frequentist performance than the alternative strictly frequentist approximation.

In order to perform inference (i.e. estimation and prediction) on spline regression smoothers, it is convenient to exploit the fact that they can be viewed as particular generalized additive models (GAM) [49, 50]. Indeed, such smoothers are GAM with only one covariate and link function between mean response variable and linear predictor equal to identity, provided that such response variable is generated from a dispersion-exponential family [50] (here we assumed the normal one). Therefore, the implementation has been performed by means of the mgcv package of R software [51], which is devoted to GAM estimation and prediction.

Finally, some normality tests (i.e. Anderson- Darling, Lilliefors and Shapiro-Wilk) on the response variable confirmed the reliability of the inferential results even for moderate sample sizes in the majority of cases (normality hypothesis acceptance rates higher than 95% for bears and higher than 50% for seals).

Results

Exposure data

A synthesis of recent data (referred to the five years period 2006-2010) available for different environmental matrices characterising the Arctic food chain is shown in Table 1. For fish, data were not readily available for some chemicals. On the contrary, for seals and bears the information was complete. This complete dataset is reported in the Supplementary Material (Tables S-10 to S-12).

For large chemical classes, like PBDEs and PCBs, the total sum reported in the different papers is often calculated on a non-constant number of congeners. However, selected congeners (e.g. Σ_4 PBDE and Σ_{10} PCB) represent a very high percentage of the total sum of analyzed congeners. Therefore, this selection represents a more reliable and comparable figure. Particularly relevant, especially for biota, are $PBDE_{47}$ and PCB_{153} [52]. The percentage of $PBDE_{47}$ is about 47% and 70% of the total PBDE concentration in fish and mammals respectively. For PCB_{153}, the values of about 10-15% of the total for fish, 20% for seal and 42% for bear, are in good agreement with those reported by Muir *et al.* [53]. Finally, it is interesting to note that the calculated concentrations in bear milk were in good agreement with the few data available in the literature [54].

Concentration values have been used to calculate BMFs in marine mammals (Table 1). Some apparently surprising data, such as the very high value for aldrin of the bear/seal BMF, may be justified by the relatively few data available for one or both animals, for some chemicals, in the considered period. More relevant is the low DDT value for bear/seal BMF indicating no biomagnification at the top level of the food chain. In this case, the value is supported by a huge amount of data from several papers referred to different arctic locations indicating that for all the cases, the concentrations in seals were higher than in bears. Possible explanations are discussed below.

Toxicity data and mixture risk characterisation

ADI for humans, assumed as reference value for seal and bear, were selected from the literature. The selected ADI and the HQs for seal and bear are reported in Table 2. Bear data sets (adults eating seals and cubs drinking milk) are complete. On the contrary, for seals, some experimental data on fish was lacking (see Table 1). Therefore, estimated fish concentrations were used as approximated exposure data (see detail of the estimation procedures in the Supplementary Material, Section 7). All the approximated values have been calculated using "worst case" approaches, so the results may be overestimated.

The following comments can be made from the results shown in Table 2.

For seals, the HI is higher than the threshold of 1. It must be considered that the assessment is based on the very conservative ADI developed for human health, and that all the assumptions used to cover the uncertainties were worst-case assumptions. Nevertheless, the value of the HI and the level of uncertainty indicate at least a situation of potential concern.

For bears, the HI is extremely high, two and three orders of magnitude higher than the threshold of 1 for adults and cubs, respectively. Even considering the very conservative approach used, the probability of toxicological risk for bears is high. In particular, taking into account that most POPs may have endocrine disrupting effects, growth and development of bear offspring may be endangered.

From the fingerprint of chemical risk in adult bears and cubs (see Figures 1 and 2), some relevant observations can be made on individual chemicals. It is worth noting that, besides the most important complex groups of POPs (PCBs, PCDD/Fs) even some individual chemicals (or small groups), such as chlordanes, aldrin and dieldrin, reach a very high level of HQ. In particular, for bear cubs some HQ values are close to or even higher than 100. Therefore, even risk from individual chemicals is high. The contribution of DDTs and its metabolites on the total potency of the mixture is relatively low. This is mainly due to the higher ADI of DDTs and, for bear cubs, to the low BMF between seals and bears.

For seals (Figure S-2 in the Supplementary Material), the highest HQs correspond to PCDD/Fs. Only PCDD shows an individual HQ higher than 1.

The most harmful individual chemical for bear cubs is PFOS, showing a substantially lower risk in adult bears. This is due to the very high BMF of this chemical from seal to bear (BMF = 34). To confirm the reliability of this value, data from the same geographic area, referred to the same period, reported in the same paper [55] show a difference of two orders of magnitude between seal and bear concentrations. The reasons for this particular pattern should be better investigated.

Temporal trend of risk

The dataset presented in Table 1 was enlarged with all data available in the literature, covering the period lasting from late 1960s to 2011. The criteria for the selection of data and their elaboration are the same as reported before. The complete data set is reported in the Supplementary Material (Tables S-10 to S-12).

Sufficient data are available for many chemicals in order to reliably characterise the temporal trends in seals and bears. In some cases, reliable data allowed to evaluate the trend for more than forty years.

The complete temporal trends, together with the statistical data of the obtained models and an estimation of the half-lives of the different chemicals, are reported in the Supplementary Material (Section 8, Figure S-3, Table S-9).

The description of temporal trends for legacy and emerging POPs is reported in several recent reports and papers in the literature, either for the Arctic as a whole [10, 16-19, 56, 58] or for specific areas, such as Greenland or the Canadian Arctic (see, for example [20-21, 59-62]). The results obtained are in good agreement with the literature data showing a general decrease of legacy POPs starting from the 1980s. On the contrary, PBDEs and perfluorinated compounds (particularly PFOS) continuously increase and only very recent data (after 2005) seem to indicate a decrease [63-70].

Based on the obtained trend models, a temporal trend of the risk for bear cubs has been calculated. It refers to the mixture of the chemicals for which enough data was available to calculate a reliable temporal trend model. Therefore, this mixture is not complete. However, it represents about 80% of the total risk calculated for the period 2006-2010. Among the excluded chemicals, only PCDD/Fs represent a relevant percentage of the risk (about 16%). The trend of the risk for bear cubs, from 1985 to 2010, is shown in Figure 3, together with the percentages of the five chemicals (or groups of chemicals) more relevant in the composition of the mixture. The following comments can be made on these results.

- The total risk is slowly decreasing with a reduction of the HI of about 30% in 25 years, but the composition of the mixture is substantially changing.
- The percentage of risk determined by some legacy POPs (chlordanes, dieldrin) is constantly decreasing while for PCBs the decrease is delayed of about 10 years; this is probably due to some higher difficulties in the control of emissions of PCBs, compared to pesticidal POPs. Moreover, the temporal trend has been calculated on the PCB153 only, because of its higher biomagnifications capacity [52]. This property could increase its persistency in biota and makes this congener particularly relevant for risk assessment.
- On the contrary, the percentage of risk determined by PFOS is strongly increasing, reaching about 50% of the total HI in 2010.

Discussion

Ecotoxicological risk

One must be aware of the approximation of the approach, based on a number of assumptions, mainly as part of the estimation of mixture response. Applying the CA model to hazard quotients calculated using estimated acceptable daily intakes for humans is an extreme simplification, particularly for a complex class of chemicals like POPs, with extremely different toxicological modes of action. However, it represents a possibility for estimating an approximated response with incomplete information available.

Another source of uncertainty and approximation is the possible variability of POP content as a function of age and sex. This could be particularly relevant in relation to the transfer of POPs to offspring during reproduction and lactation. Aguilar and Borrell [71] estimated the reproductive transfer of organochlorine pollutants in the offspring of fin whales (*Balaenoptera physalis*). They observed a decrease of DDTs and PCBs in adult females, probably due to excretion during reproduction and lactation. Therefore, they calculated that the total intake to the offspring trough lactation was about 1 g of PCBs and 1.5 g of DDTs for primiparous females, decreasing to 0.2 g of PCBs and 0.3 g of DDTs for old females. This kind of detail was not the objective of this paper. However, for some chemicals (e.g. chlordane, DDTs, PCBs) and some temporal intervals, it has been possible to get separate data for male, female and sub-adult bears (see the database in Supplementary Material). The observed differences were, in any case, lower than a factor of two, assumed as irrelevant for an approximated assessment.

Aguilar and Borrell [71] concluded that considering the size of the fin whale, the toxicological risk is low. Indeed, the body weight of a newborn fin whale is about 2 metric tons, the lactation time-span is at least 6 months and the weight at weaning is more than 10 metric tons [72]. For a polar bear cub in the first 40 weeks of life (body weight growing from about 0.6 to about 80 kg), a comparable calculation indicates a total intake of about 4 g of PCBs with an average body weight of about 38 kg for the 40 week interval (see details of the calculation in Supplementary Material, Section 7, Table S-8). This shows the enormous difference in risk between whales and polar bear, mainly due to the different position in the food web.

The described assessment indicates a very high potential for toxic effects at least at the top levels of the Arctic food web, particularly for a top predator like polar bear and for its offspring. Moreover, it must be noted that, besides the effect of the mixture, for four chemicals (or group or chemicals) the risk for the most endangered organisms of the food web (bear cubs) is two orders of magnitude higher than 1 , assumed as the threshold of risk (see Table 2).

We are aware of the approximation and uncertainty of the assessment due to a series of factors (geographic variability, sex, age and diet differences, etc.). Nevertheless, a risk orders of magnitude higher than a safety threshold overcomes these uncertainties. Therefore, the results represent a serious warning for the risk from POPs for the Arctic ecosystem. In particular, it is recognised that most POPs are endocrine disruptors. Endocrine disorders in polar bears were described by Wiig *et al.* [73] who observed cases of pseudo-hermaphroditism in females sampled at Svalbard in 1996. The Authors hypothesized that it could be an effect of POP contamination.

Extensive reviews [74, 75] highlight the occurrence of several health effects in Arctic top predators, particularly in polar bears, that may be attributed to POPs. Some examples are reported in table 3, but the list is far to be exhaustive. Considering the estimated risk of POP mixture, particularly for bear cubs, these evidences of health effects are not surprising.

Differences among individual chemicals

Individual chemicals play a different role in the composition and fingerprint of the mixtures, both as concentrations and as toxic effects on different organisms of the food chain (Tables 1 and 2 and Figures 1 and 2 of the main text; Tables S-5, S-6, S-7 and Figure S-2 of the Supplementary Material). It may be observed that a few chemicals cover a large percentage of the total potency of the mixture. In particular, for adult bears five chemicals or chemical classes explain about 90% of the total mixture potency: PCDD>PCDF>toxaphene>dieldrin>chlordanes. Completely different are the most toxic chemicals for bear cubs for which about 90% of the total mixture potency is explained by other five chemicals or chemical classes: PFOS>PCB>chlordanes>PCDD> dieldrin. In particular, PFOS alone is responsible for about 50% of the toxic potency. Indeed, PFOS concentrations are relatively low in seals and dramatically increase in bears and, as a consequence, in bear milk. Moreover, PFOS is considered very toxic for mammals, with a very low ADI [94]. However, the sequence must be taken with care, considering that the exposure to some of the chemicals was calculated with approximated procedure.

Among chlorinated insecticides, DDT plays a relatively lesser role in the mixture, in spite of the extremely high global emissions, estimated in the range between 1.2 and 4.1 million of metric tons [95]. This is partly due to the relatively low toxicity for mammals in comparison with other chlorinated insecticides (ADI two orders of magnitude higher than those for aldrin, dieldrin and chlordane are are).

Moreover, the relatively low concentrations in adult bears, substantially lower than in seals, indicate no biomagnification in bears. This justifies the low HQ for bear cubs.

The low values of DDTs in polar bear could be explained by a capacity of bear to metabolize DDTs more efficiently that other POPs. This capacity should be typical for bears and not for seals. This kind of metabolic capability was already observed by Norstrom *et al* [56] and by Letcher *et al* [27]. Polischuck *et al.* [54] observed that that polar bears are able to metabolize DDTs more readily than most organochlorine compounds and that they appear to be unique in the animal world in their capability to metabolize *p, p'*-DDE. Letcher *et al*. [27] measured BMF between seals and bears for DDTs and PCBs of 0.6 and 15.1 respectively. These values are in reasonable agreement with those calculated as 0.5 and 24 respectively in our dataset (Table 1).

Risk trend and effectiveness of control measures

Since some decades, the global emissions of legacy POPs are dramatically reduced due to several international agreements such as the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal signed in 1989 [96], the Aarus Protocol on Long Range Transboundary Air Pollution (LRTAP) of POPs, signed in 1998 [97] and the Stockholm Convention, signed in 2001 and entered into force since 2004 [5]. In particular, the last two agreements are based on an original list of chemicals to be controlled. This list is periodically amended and new chemicals are included. The list of chemicals controlled to date by the Aarus Protocol and by the Stockholm Convention is reported in the Supplementary Material (Table S-1).

To evaluate the effectiveness of the Stockholm Convention, a complex strategy has been developed based on a global monitoring plan, national reports, and the measures taken to implement the Convention and on other initiatives under the control of the Stockholm Convention Conference of the Parties [98]. A full evaluation of the effectiveness of the Convention is planned for the year 2017.

The effectiveness of the control measures is supported by the evidence of the decreasing trend in the Arctic ecosystem starting from the late 1980s. However, the decrease is very slow. For individual compounds, half-lives in the order of years to decades are reported in the literature (see for example [99]). Comparable values have been calculated with the models developed in this work (half-lives ranging from 4 to 96 years for the different chemicals, see table S-9).

A relationship between the trends in biota (seals and bears) and the trends in the surrounding environment (water) is not easy to find because water data are scattered and often with poor comparability among data from different surveys. However, Choi and Wania [100] have theoretically demonstrated the possibility of a very slow environmental reversibility for some classes of POPs in conditions of relatively long air and water half-lives, likely to occur in polar environments. This reversibility may last far beyond the middle of this century.

Moreover, the concern is growing for other chemicals, already listed as POPs under the Stockholm Convention since 2009, but for which a decrease started recently or is not yet effective, such as PBDEs and, particularly, PFOS. The concentrations of PFOS in polar bears are surprisingly high (two orders of magnitude more than in seals) and are reason for a growing concern. At present, for polar bear cubs, PFOS represents the most harmful chemical in the mixture (Figure 2).

The need for improving knowledge on temporal trends of legacy and new POPs in biotic and abiotic matrices of the Arctic is highlighted by Muir and de Wit [58], also considering possible effects of climate change on ecological characteristics of the system and on fate patterns of the chemicals.

Climate change may alter contaminant pathways and concentration patterns as well as the vulnerability of fragile ecosystems with processes that still remain far from a complete understanding and predictability [101].

The number of POPs circulating to date in the global environment is quite controversial. Brown and Wania [102] examined a data set of more than 100,000 chemicals and identified 120 high production volume chemicals, which have properties that suggest they are potential Arctic contaminants. Their list included several current use pesticides as well as halogenated chemicals that have not been measured previously in the Arctic. Indeed, recently, Morris *et al.* [103] report evidence of the presence of current-use pesticides in the Arctic food chain. Scheringer *et al*. [104] examined a data set of more than 90,000 compounds and found 510 chemicals exceeding the criteria to be considered potential POPs. Considering the uncertainty of the screening exercise, the authors conclude that at least 190 chemicals may be considered potential POPs and that several tens of potential POPs may have to be expected for future evaluation under the Stockholm Convention. At present, the Stockholm Convention includes 23 chemicals (or congeneric groups) and 6 candidates.

Another relevant issue concerns chemicals that are unintentionally produced as by-products of industrial processes (e.g. PCDD/Fs). For these chemicals a complete phasing out is virtually impossible and only a reduction of emissions is realistically achievable, at least in the short time.

Conclusion

In the last few decades, a huge amount of papers and technical reports were published on the presence and temporal trends of contaminants, particularly POPs, in the Arctic environment. Many of them focus on specific groups of chemicals, specific matrices or animal species, specific locations. Others are more general providing a wider picture of Arctic contamination. In a few cases, attempts are made to assess the general toxicological impacts of global emissions of contaminants of human origin (see, for example, [23]). However, even if the occurrence of adverse effects is highlighted, no attempts are made for a quantitative characterisation of the risk.

The objective of this work is not repeating what is already present in the literature but using the bulk of information available in order to develop a quantitative characterisation of the ecological risk determined by a mixture of high volume POPs, likely to occur in the Arctic environment as a whole.

The results of this work are based on a series of assumptions and approximations needed because of the lack of a complete and detailed knowledge on many aspects of the process of risk characterisation. In particular, the complexity of the toxicological modes of action of the chemicals considered leds to assume, as toxicological endpoint, not a specific effect but a conservative reference value like the ADI. For the same reason, the only possibility to estimate the response to the chemical mixture has been the use of the Concentration Addition model. All these assumptions led to a worst-case characterisation and to a possible overestimation of the actual risk. Nevertheless, the extremely high level of the risk and the very slow reduction estimated in the last twenty years, in spite of the effective control measures indicates that POPs represent a serious environmental concern at the planetary level. Moreover, the observed changes in the composition of the mixture highlight that growing attention must be paid to emerging contaminants. These results also provide insights for international stakeholders for the need for a further implementation of mitigation measures, such as the Stockholm Convention, for legacy as well as for additional, not yet controlled POPs, in order to avoid global pollution problems that will require generations to be solved.

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Appendix A. Supplementary data

Supplementary data to this article can be found online.

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Figures

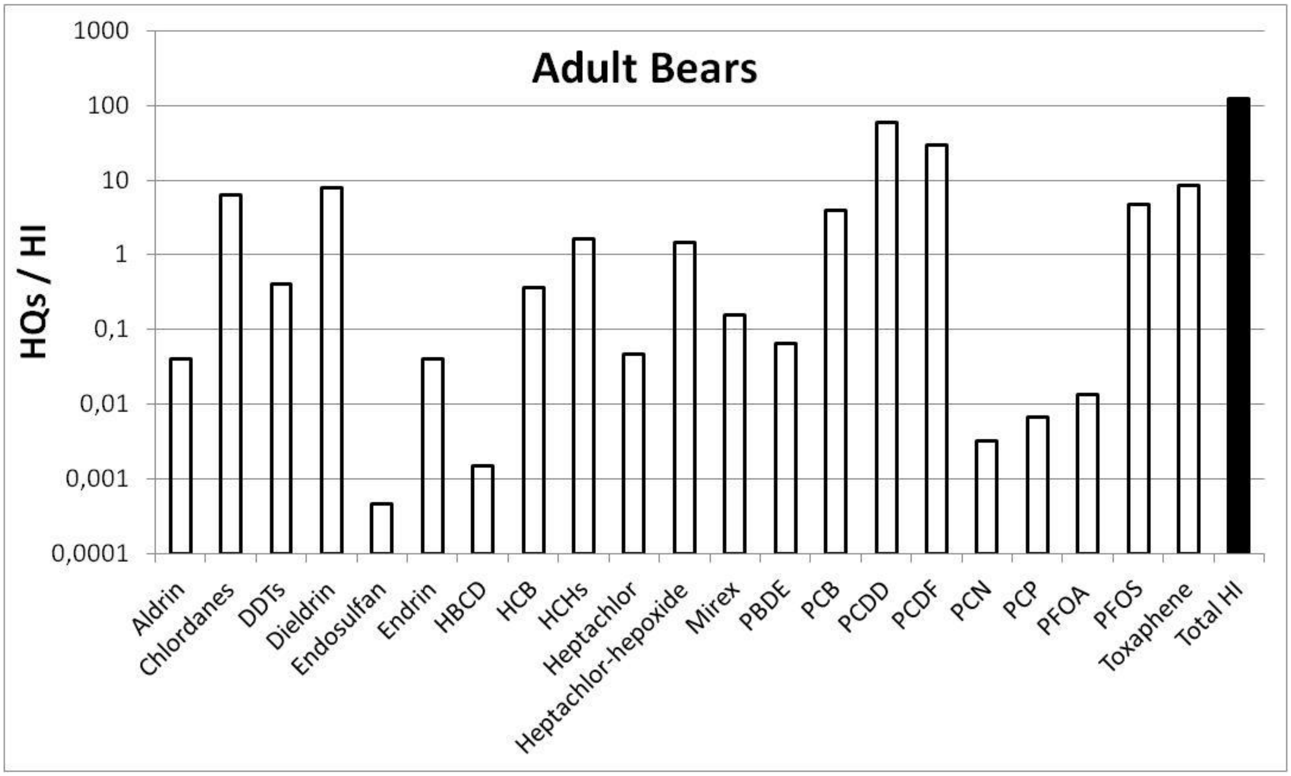

Figure 1– HQs calculated for individual contaminants and HI for the total mixture indicating the risk for adult bears due to biomagnification of POPs in the Arctic food web.

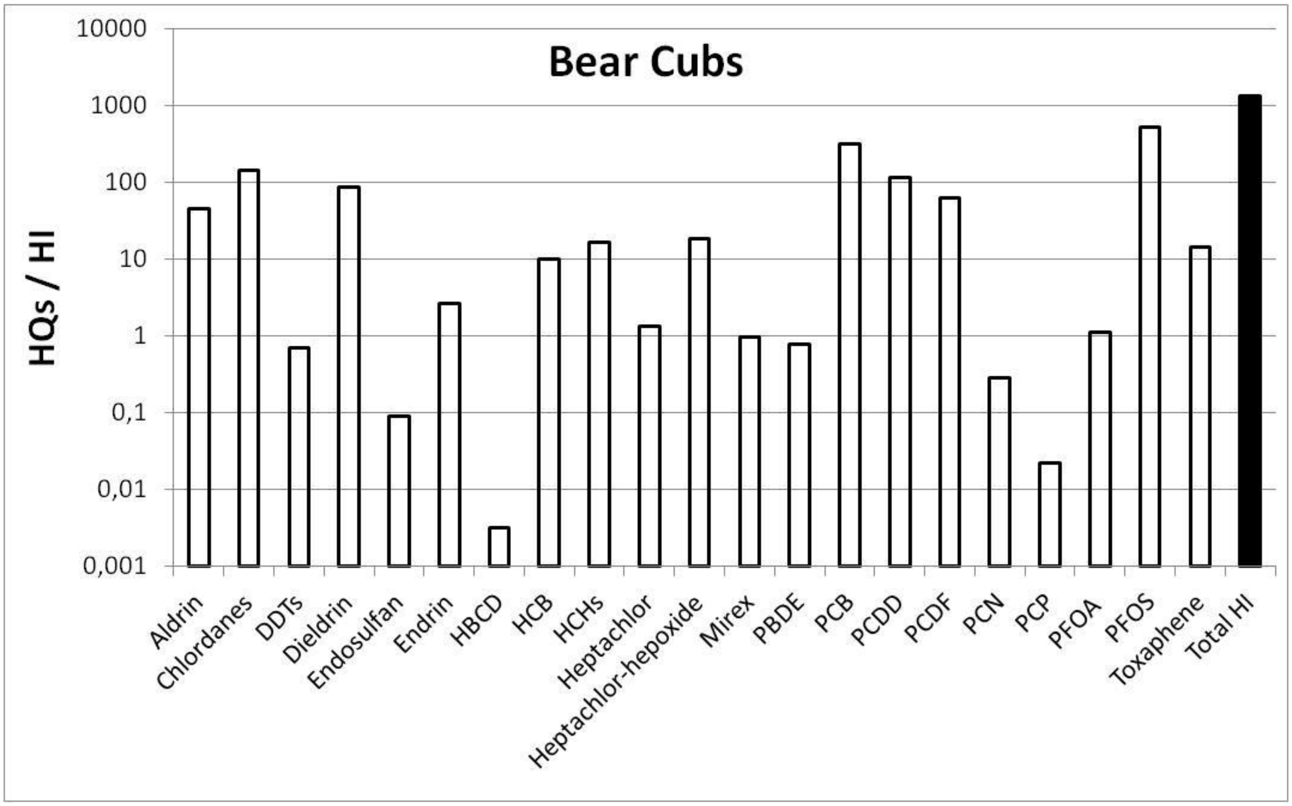

Figure 2 – HQs calculated for individual contaminants and HI for the total mixture indicating the risk for bear cubs due to POP contamination of milk.

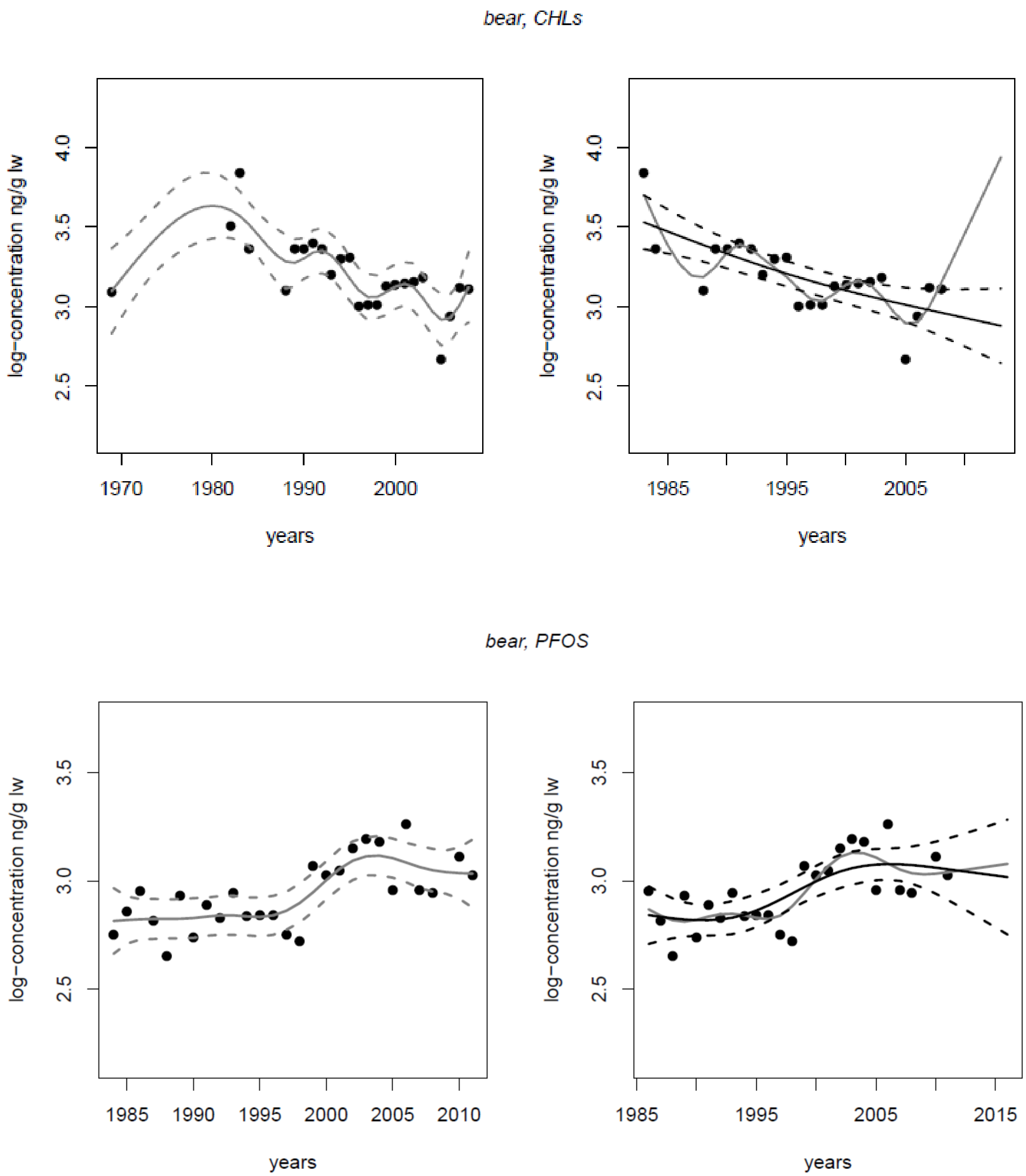

Figure 3 – Examples of spline penalized regression smoothers of concentrations in bears for chlordane (above, ng/g lipid weight) and PFOS (below, ng/g wet weight). Plots on the left (grey solid lines) cover the whole period, plots on the right refer to more recent data (grey solid lines for best model, black solid lines for 4 knots (i.e. trend) model, dotted black lines for 5 knots model when present). Dashed lines represent 95% confidence limits. The complete set of temporal trends is reported in the Supplementary material (Figure S-3).

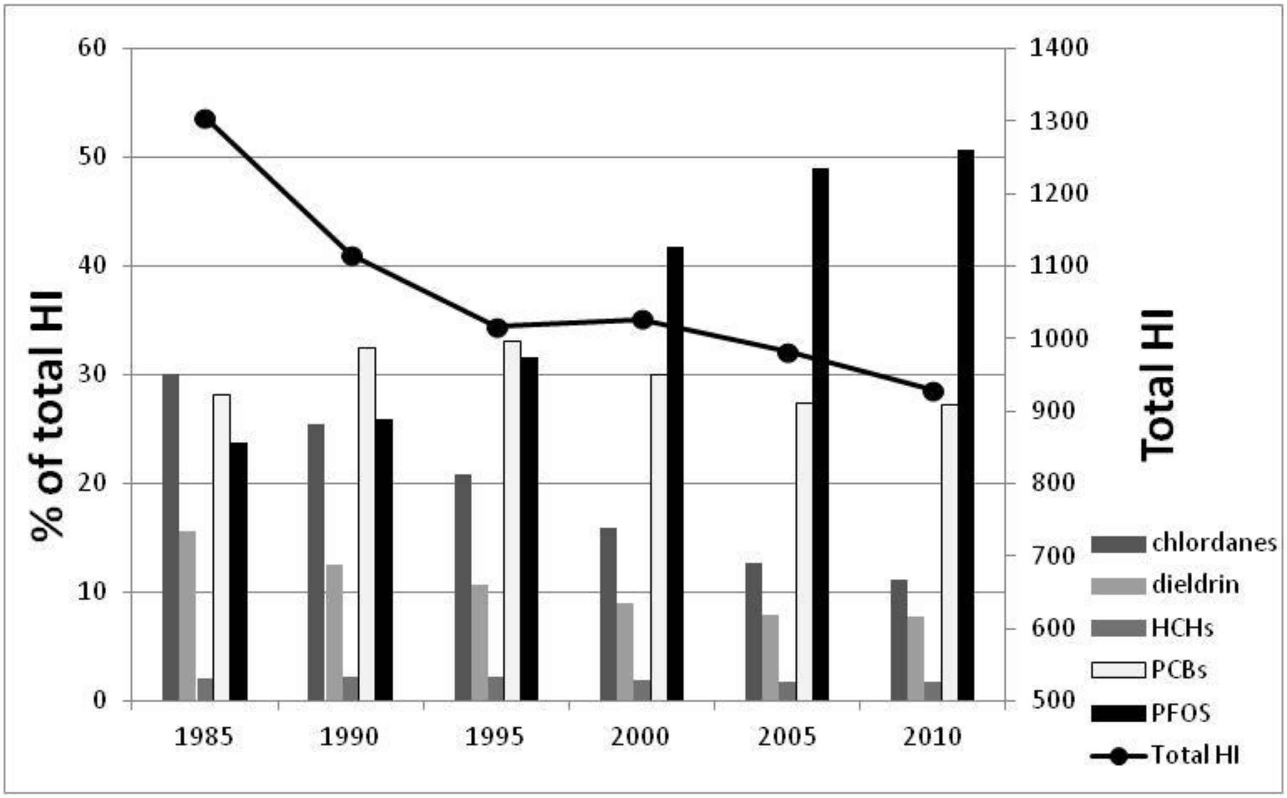

Figure 4 – Temporal trend of the POP mixture risk for polar bear cubs (line, axis on the right) and percentages of the five more relevant components of the mixture (histograms, axis on the left).

Tables

Table 1. List of selected chemicals used in the risk assessment. Details on their properties are in the Supplementary Material.

Individual chemicals and small groups	***Large chemical groups***
aldrin	polychlorinated biphenyls (PCBs)
chlordanes	polybrominated biphenyl ethers (PBDEs)
DDTs	polychlorinated dibenzo dioxins (PCDDs)
dieldrin	polychlorinated dibenzo furans (PCDFs)
endosulfan	polychlorinated naphtalenes (PCNs)
endrin	perfluorinated compounds (PFOA, PFOS)
heptachlor and heptachlor hepoxide	
hexabromocyclododecane (HBCD)	
hexachlorobenzene (HCB)	
hexachlorocyclohexanes (HCHs)	
mirex	
pentachlorophenol (PCP)	
toxaphene.	

Table 2. Selected concentrations of the considered POPs in biota (n.d. = not detected; n.a. = data not available) referred to the period 2006-2010 (see materials and methods). Data in italics refer to the previous five years period (2001-2005). Unless differently indicated, concentrations in biota are normalised to lipid weight (lw). Lipid content has been assumed as 7% in fish [36], 93 and 87% in seal and bear fat, respectively (geometric mean of data reported in the Supplementary Material). Data are weighted geometric means from data collected as described in the method section. Biomagnification factors (BMF) .between cod and seal and between seal and bear re also reported.

	Polar cod	**Ringed seal**	**Polar bear**	**Bear milk (calc.)**	**Bear milk (measur.)**	**BMF**	
	ng/g lw	ng/g lw	ng/g lw	ng/g ww	ng/g ww	**Seal/Cod**	**Bear/Seal**
Aldrin	n.a.	*0.2*	*70*	*23*		n.a.	389
Chlordanes	20	160	1080	356	910	80	6.8
DDTs	*50*	206	106	35	44	4.1	0.5
Dieldrin	*8.7*	39	133	44		4.5	3.4
Endosulfan	*2.9*	*0.14*	*8.1*	*2.7*		0.05	58
Endrin	n.a.	*0.4*	*8.0*	*2.6*		n.a.	20
HBCDs	*3.1*	*7.6*	4.8	1.6		2.5	0.6
HCB	11	7.5	92	30		0.68	12
HCHs	5.1	82	253	84	85	16	3.1
Heptachlor	*0.02*	*0.23*	*2*	*0.66*		11	8.7
Heptachlor-hepoxide	*4.3*	37	139	46		8.6	3.8
Mirex	52	*3.9*	7.4	2.4		0.08	1.9
PBDE [1]	4.3	6.6	24	7.9		1.5	3.7
Σ_4 PBDE [1]	4						
PCB [1]	29	197	4741	1564		16	24
Σ_{10} PCB [1]	*22*	*618-*	*2782*	*916*	780		
Σ_7 PCDD [2]	n.a.	*0.008*	*0.044*	*0.015*		6.6	5.5
Σ_7 PCDD [3]	n.a.	*0.006*	*0.0035*	*0.0012*			
Σ_{10} PCDF [2]	n.a	*0.020*	*0.012*	*0.004*		n.a.	0.6
Σ_{10} PCDF [3]	n.a.	*0.003*	*0.0019*	*0.0006*			
PCN	n.a.	*0.16*	*4.3*	*1.4*		n.a.	27
PCP	n.a.	*1*	*1*	*0.33*		n.a.	1
PFOA [4]	0.17	1	25	13		5.9	25
PFOS [4]	1.5	35	1182	591		23	34
Toxaphene	n.a.	85	43	14		n.a.	0.5

1. For PBDEs and PCBs, different congener selections are reported. More explanations provided in the Supplementary Material.
2. For PCDDs and PCDFs, 7 and 10 coplanar congeners respectively are reported.
3. As toxic equivalent quotient (TEQ).
4. For perfluorinated compounds the concentrations are expressed as whole body w.w. (cod) liver ww (seal and bear).
5. Measured data [49] referred to 1992-95.

Table 3. Selected ADI of the considered POPs and calculated HQs for seal nd bear. Data in italics are calculated using estimated exposure values. Details on the origin of data and references as well as on the estimation procedures are reported in the Supplementary Material (Section 5, Tables S-3 and S-4).

	ADI	**HQs**		
	mg/kg bw day	**Seal**	**Bear (adults)**	**Bear (cubs)**
Aldrin	0.0001	*0.005*	0.04	46
Chlordanes	0.0005 [1]	0.20	6.4	142
DDTs	0.01 [1]	0.025	0.41	0.7
Dieldrin	0.0001	0.43	7.8	88
Endosulfan	0.006	0.0024	0.0005	0.09
Endrin	0.0002	*0.0098*	0.04	2.6
HBCDs	0.1	0.0002	0.0015	0.003
HCB	0.0006	0.06	0.37	10
HCHs	0.001	0.025	1.6	17
Heptachlor	0.0001	0.001	0.05	1.3
Heptachlor-hepoxide	0.0005	0.042	1.5	18
Mirex	0.0005	0.51	0.16	1.0
PBDEs	0.002	0.011	0.066	0.8
PCBs	0.001	0.14	3.9	313
Tot. PCDDs	2E-09 [2]	*1.4*	60	115
Tot. PCDFs	2E-09 [2]	*0.66*	30	63
PCNs	0.001	*0.0004*	0.003	0.28
PCP	0.003	*0.0001*	0.007	0.02
PFOA	0.0015	0.0006	0.013	1.1
PFOS	0.00015	0.049	4.7	520
Toxaphene	0.0002	*0.25*	8.5	14
Mixture HIs		**3.81**	**126**	**1355**

[1]Provisional

[2] As 2,3,7,8-TCDD equivalents (TEQs)

Table 4. Some evidences for health effects in polar bears

Observed effect	Chemicals involved	References
Activity of Cytochrome P-450 and associated enzymes	Several groups of POPs (e.g. PCBs, PCDD/Fs)	[71], [72]
Alteration to the endocrine system	Several groups of POPs (e.g. PCBs, PBDEs, HCB, HCHs and DDTs)	[73], [74], [75], [76], [77], [78], [79], [80]
Malfunctioning of reproductive organs	Several groups of POPs	[81], [82], [83], [84], [85]
Liver alterations	Several groups of POPs	[86], [87],
Neurological damages	Several groups of POPs	[88]