MEETING ABSTRACTS

Environmental contaminants and animal health. Proceedings of the 26th Symposium of the Nordic Committee for Veterinary Scientific Cooperation (NKVet)

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FOREWORD

S1 Foreword: Environmental contaminants and animal health. Proceedings of the Nordic Committee for Veterinary Scientific Cooperation (NKVet)
Raimo Pohjanvirta
Chair of the Scientific Programme Committee for the 26th NKVet Symposium, Department of Food Hygiene and Environmental Health, University of Helsinki, Helsinki, Finland
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The 26th NKVet (Nordic Committee for Veterinary Scientific Cooperation) Symposium was held in Helsinki, Finland, at 6–7.10.2011. The title of the Symposium was “Environmental Contaminants and Animal Health” and the focus was on the insidious health risks posed to both humans and animals (wild & domestic) by the often ubiquitous synthetic and biological environmental chemicals. The scientific programme consisted of five overview-type keynote speeches along with a total of twelve invited talks on specific topics. The presentations addressed the “classical” but still today highly pertinent environmental toxicants such as dioxins, PCBs and other persistent organic compounds; emerging contaminants such as diverse endocrine disruptors; biological toxins such as mycotoxins; and up-to-date biological assay methodologies available for detection and quantification of these environmental hazards. This special issue of Acta Veterinaria Scandinavica contains abstracts or full-length papers of the presentations given in the Symposium. The interested reader should notice that even for most of those presentations of which only the abstract is included here, the original slides (in pdf-format) can be found at the NKVet web site (http://www.nkvet.org/index.php?id=11).

MEETING ABSTRACTS

S1 Male reproductive health as a sentinel for environmental endocrine disruption
Jorma Toppari
Departments of Paediatrics and Physiology, University of Turku, FI-20520 Turku, Finland
E-mail: jorma.toppari@utu.fi
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Effects of endocrine disruptors on experimental animals and wildlife are well established. Antiandrogens and estrogens cause reproductive disorders, such as cryptorchidism, hypospadias, testicular dysgenesis and subfertility. This raises a natural question whether these disorders in humans are also caused by endocrine disrupters and whether they should alert us of harmful exposures. While we still do not know the answer to this question, we know that the incidence of testicular cancer has rapidly increased over two generations, and the birth rates of hypospadias and cryptorchidism are alarmingly high. Furthermore, semen quality of young European men remains very poor at the moment. We have analyzed the association of cryptorchidism with exposure to several endocrine disruptors. This kind of studies cannot prove any causality. However, we have found a weak positive association of cryptorchidism with exposure to chlorinated pesticides, dioxins and furans, and polybrominated diphenyl ethers. It has become obvious that there is no individual compound that could be linked to etiology of cryptorchidism, but rather a mixture of several chemicals can cause the effect in genetically susceptible individuals. Modern systems biological approaches are needed to deal with complex exposure scenarios and genetic variability.

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S2 Anthropogenic pollutants – an insidious threat to animal health and productivity?
Stewart M Rhind
The James Hutton Institute, Craigiebuckler, Aberdeen, AB15 8QH, UK
E-mail: stewart.rhind@hutton.ac.uk

Introduction: Pollution is a natural phenomenon. For example, crude oil constantly leaks from below the Earth’s crust in many areas, volcanic outputs include multiple toxic gases and forest fires result in the production of toxic hydrocarbons. However, both the amounts and range of types of pollutants released into the environment have been greatly increased by human action since metals were first smelted, thousands of years ago, increasing environmental levels of heavy metals [1]. The increases have been particularly marked during the last 60 years, as many new organic chemicals have been synthesised and used in thousands of...
products. The recently-synthesised compounds are chemically-diverse in nature, each having been created to have certain properties e.g. organochlorine compounds such as DDT are highly effective insecticides, phthalates are used as softening agents in plastics, polychlorinated diphenyl ethers (PBDEs) are effective fire retardants used in electrical equipment and furnishings, and polychlorinated biphenyls (PCBs) were used, formerly, as coolants in electrical equipment [2]. Some others are by-products of combustion of the fossil fuels essential for modern lifestyles (polycyclic aromatic hydrocarbons; PAHs) [3,4]. All are found in the environment, although generally at very low concentrations.

The significance of this change in the nature and amount of pollutants lies in the fact that many of these chemicals perturb animal physiology, even although in most cases they are entirely synthetic and there is no naturally-occurring equivalent. Many of these compounds can bind to cellular receptors or otherwise interfere with hormonal signalling and enzyme systems in species as diverse as bacteria [5] and mammals [6]. Consequently, they have the capacity to disrupt normal endocrine function and are collectively described as endocrine disrupting compounds (EDCs). In addition to these organic pollutants, elemental pollutants, such as lead and mercury [7] also contribute to the pollutant burden and associated adverse physiological effects, sometimes acting in concert with organic pollutants [8].

Additional classes of anthropogenic compounds implicated in disruptive effects include some used to directly enhance animal and human health (analogic pharmaceuticals; [9]) and crop production (nitrate fertilisers; [10]). All of these pollutants have become ubiquitous in the environment.

**Laboratory investigation of mechanisms and effects:** Arguably, issues of environmental pollution and animal and human health first achieved prominence with the publication of Silent Spring [11]. This highlighted the effects of some of the new chemicals on wildlife and probably stimulated some of the many detailed studies of effects and mechanisms of action. Many of the studies designed to elucidate mechanisms of action have involved laboratory rodents subjected to high level, short-term, exposure to single chemicals. Such studies have clearly shown that such exposure could induce structural and physiological changes in the reproductive system [12,13] and could compromise immune function [14,15]. Such studies also contribute to understanding of the underlying mechanisms of action of EDCs. In parallel with the laboratory studies, field observations of a wide range of species showed further evidence of adverse physiological effects, particularly in relation to reproduction [16,17] with associated effects on populations [18]. However, many of these observations concerned abnormally high levels of exposure associated with accidental releases of pollutants or with other unusual patterns of exposure (e.g. employment in factories where EDCs were used in manufacturing processes). Perhaps unsurprisingly, concerns about anthropogenic environmental pollutants remained relatively muted for some time because both field observations and laboratory studies involved high levels of single chemicals, making, it is easy to dismiss the observed effects as irrelevant in most circumstances. Environmental (low) concentrations of these compounds were seldom associated with apparent adverse effects on either animals or humans. Furthermore, many of the compounds concerned were of great value to crop production and disease control (herbicides and pesticides) or contributed to more comfortable (electrical equipment) or safer lifestyles (fire retardants) and the will to investigate possible adverse effects may have been limited.  

"Real world" exposure patterns are different: It is often suggested that low concentrations of environmental pollutants are of no concern because, in most circumstances, concentrations are below the No Observed Effect Levels (NOEL); i.e. environmental levels of each individual chemical have been shown to be below the minimum concentrations known to induce a physiological response. However, this argument fails to take account of several factors:

a) **Mixtures:** It is now well recognised that EDCs act additively [19,20] and, possibly, synergistically [21] on physiological systems i.e. the combined effect of multiple EDCs, even at concentrations too low to induce a response, can induce adverse effects.

b) **Exposure differs with age and stage:** It is often assumed that exposure to EDCs is similar in adult and fetal / juvenile stages of development and that they are subject to equivalent pollutant insults. In fact, studies of sheep have shown that fetal tissue concentrations of most EDC classes are lower than those of their dams, although some can be preferentially accumulated in fetal tissues [22,23]. This is presumably as a result of a shorter period of exposure in the fetus and differences between the fetus and dam in uptake, metabolism and excretion. In some animal groups, the insult may differ with stage of development because food sources, and therefore pollutant exposure patterns, differ e.g. immature ruminants feed on milk while mature animals are herbivorous; similarly, developing poultry derive nutrient from egg yolk while adults have very different food sources. These differences make it difficult to extrapolate tissue burden data across animals at different stages of development or to determine a critical level of exposure.

c) **Sensitivity differs with age and stage:** The occurrence of lesser tissue EDC burdens in young animals might be assumed to be associated with a lesser risk of disruption. However, like exposure rate, susceptibility to disruption by environmental pollutants differs with age being greater during early developmental stages [24]. Studies of sheep have shown that exposed fetuses exhibit disrupted development even although some equivalent effects are absent in their dams [25,26]. Thus, the NOEL is different for animals at different stages of development.

d) **Individuals and species differ:** Individual animals exhibit a very high degree of variation in the rate of tissue accumulation of EDCs even when apparently exposed in an identical way; this presumably reflects differences between individuals in uptake, metabolism and excretion [2].

Consequently, it should be noted that the capacity to metabolise pollutants is highly dependent, also, on species [27] and so extrapolation of the NOEL between species requires caution.

**Farmed animals:** Initial concerns about EDCs were centred, primarily, on terrestrial, vertebrate wildlife, because it was in wild animals that adverse effects were first noted, but subsequent research has implicated EDCs in effects on farm animals [2], marine, freshwater and terrestrial invertebrates [28-30] and humans [31,32].

Modern husbandry practices and modern household environments are both associated with increased exposure to the ubiquitous EDCs through food, water, inhaled air and administered pharmaceuticals [33]. Interest in environmental pollutants with respect to animals reared for food or as pets lies in many different areas. Consumers of farm animal products are often concerned about possible health risks associated with contaminated meat and dairy products while producers are concerned with both the image associated with their products and its monetary value. Pet owners have concerns about their animal’s health and welfare. Consequently, veterinarians have an interest in all of these issues and species.

One aspect of exposure of farm animals that may change in the future is related to the increasing scarcity and cost of oil and phosphate, both of which are essential for the production of inorganic fertilisers; the increasing cost of such fertiliser, together with concerns about pollution, is increasing the pressure to recycle food waste, green waste and sewage sludge to land. All of these recycled products provide valuable plant nutrients but both field observations and laboratory studies have shown that these materials have divergent views with regard to the importance, in relation to animal productivity and health, of prolonged, low level, exposure to these pollutants following application of waste to land. In an extensive review, Smith [34] suggested that the application of sewage sludge to land was of little concern with respect to soil, animal or human health because rates of transfer into plants and animals were likely to be insignificant. Indeed, studies of tissue levels of several classes of EDCs (phthalate, PCBs, PBDEs and PAHs) suggest that exposure of sheep to pastures fertilised with sewage sludge resulted in minimal increases in milk or tissue concentrations of these compounds, relative to control animals exposed to inorganic fertilisers [35-38]. However, numerous, subtle, adverse effects have been observed in the same sheep [39]. While exposed animals appeared entirely normal, superficially, they exhibited many adverse changes in underlying physiology which had the potential to compromise reproductive performance. Specifically, exposure to sludge-treated pastures and associated EDCs has been shown to be associated with i) perturbed activity of several fetal hypothalamic neurotransmitter pathways, which is critical for fetal Leydig and Sertoli cell numbers [40] and associated germ cell numbers in the adult [41] and ii) increased fetal ovarian oocyte expression of the pro-apoptotic protein BAX and altered expression of many other fetal ovarian proteins [42]. In addition, preliminary data indicate that there may be disruption of maternal mammary structure [43] and altered protein expression in the fetal uterus [44].
Perturbation of non-reproductive systems has also been reported with sludge exposure being associated with reduced numbers of fetal thyroid follicles and reduced maternal T3 and T4 concentrations [45] and changes in offspring behaviour [46] and adult bone structure [47,48]. While not measured in the sheep studies, in other species effects of EDC exposure on obesogenic systems [49,50] and cardiovascular function have been recorded [51,52] and it is possible that similar disruption could occur in sludge-exposed ruminants or other animals exposed to an enhanced EDC insult.

In view of these observations, it might be expected that there would be evidence of reduced reproductive success in farmed animals as a result of increased environmental exposure to EDCs. At this time, evidence is scarce. Meijer et al. [53] reported a small reduction in fertility and milk production in dairy cows exposed to sewage-contaminated water and attributed the effect to the pollutants present. The high-yielding dairy cow has been the subject of much research because she exhibits a long-term decline in fertility but the underlying causes of this decline are not well understood. Nutritional and genetic factors have been implicated [54] but while, undoubtedly, they are involved, environmental pollutants may be acting in conjunction with them to exacerbate the decline in fertility through additive, subtle effects on gene expression and/or disruption of endocrine signals. The observations of Meijer et al. [53] may simply be concerns to a more general, subtly invisible, effect of chronic, low level, environmental exposure to EDCs.

With regard to male animals, a decline in semen quality might be expected in the light of increased environmental exposure to EDCs and the effects on testis structure and function described above. One study of several farm species showed no reduction in sperm counts over a period of six decades [55]. However, it should be noted that the animals studied were selected for high fertility and may not be representative of the normal population. Furthermore, domestic ruminants store sperm and so may appear to have a high sperm count even when sperm production is reduced. Another study of bull semen [56] appeared to indicate a temporal decline in semen quality during the 1970s with an associated, anomalous improvement in sperm morphology and motility. It was concluded that there were some methodological inconsistencies which partially compromised the interpretation of the results but it was also concluded that the decline could not be readily linked to EDC exposure since semen quality subsequently improved. It seems likely that the issue is complex; it may involve environment/genotype interactions and a comprehensive understanding of the effects of environmental levels of EDCs on male fertility, or lack of them, may be some time off.

To date, little work has been done concerning effects of EDCs on domestic poultry but there is little reason to doubt that their physiology may be affected, also, since studies of wild birds have shown adverse effects of various EDCs on aspects as diverse as egg shell formation and embryo survival [57,58] and brain function, as indicated by altered song patterns [59].

In holarctic regions, farming of animals for fur is common; these species (e.g. mink, arctic fox, etc) are carnivores, near to the top of the food chain, and accumulate relatively high concentrations of pollutants in their tissues; they too exhibit adverse effects on embryo and offspring survival when exposed to specific dietary EDCs [59].

Finally, many species are farmed throughout the world; some of them are exposed to higher rates of pollutant exposure than wild fish [60] because they are fed on products containing other fish and associated accumulated pollutants. While most concern is focussed on potential effects on human health, such elevated tissue burdens may have physiological/health consequences for the fish themselves. Consumers are concerned about tissue EDC accumulation but veterinarians may have concerns about potential reproductive or immuno-suppressive effects of EDC burdens. While there do not appear to be significant concerns about such effects, to date, it should be noted that effects of environmental pollutants on reproductive physiology [61] of wild fish species have been reported indicating potential susceptibility. Domesticated newts, in the sheep studies, and their companion animals to EDCs, as carnivores, dogs and cats are exposed to EDCs in their food [62]. Also, frequently they live indoors where EDC concentrations tend to be elevated relative to outdoor air [63] and so, like humans, they are exposed to a wide range of household EDCs, in part, owing to their close proximity to the ground where they are exposed to soil and house dust into which some EDCs such as PBDEs may leach [64] and because they have a tendency to consume items other than conventional foodstuffs. To date, such animals have been studied little but, as with domestic animals and humans, economic interests may cause this to change if effects are demonstrated.

Other commercially important species: While not, typically, the subject of conventional veterinary treatments, the commercial importance of some invertebrate groups should not be forgotten. Honey bees are clearly of great economic importance and populations in Europe and North America appear to be under threat from a combination of factors, possibly including exposure to endocrine disrupting, agricultural chemicals [65]. Other invertebrate groups of commercial interest include crustaceans and freshwater snails. Much of the interest in freshwater has concerned effects of EDCs in sewage on fish populations but lower profile, invertebrate species are also affected. For example, freshwater pearl mussels, already under threat from over-exploitation, are further compromised by exposure to the anti-depressant drug fluoxetine (Prozac) present in sewage effluent discharged into rivers; exposure causes premature release of their larvae, with associated reductions in their survival [66]. Effects of tributyl tin, an anti-fouling agent used in marine paints, has long been recognised as a potent disruptor of dogwhelk reproduction [67] but larval stages of bivalve molluscs, some of which are significant commercial species, are highly sensitive to this EDC [68]. While some invertebrate species of commercial interest have a higher profile and are the subject of greater management and veterinary inputs, the commercial and biological significance of many lowly species should not be underestimated and neither should the potential adverse effects on them of environmental pollutants.

Understanding EDC actions: As indicated above, exposure of both species and individuals to pollutants is highly variable, depending on environment, diet and veterinary medical treatments, and multiple factors can influence the animals’ responses. The occurrence of disruptions of reproduction and health that can be clearly attributed to the effects of pollutants is rare, reflecting the fact that effects are generally subtle, involving changes in gene expression and the structure and function of internal organs but not gross changes in health or reproductive performance. However, such subtle effects can be greatly exacerbated in animals subject to additional stressors; thus prediction of responses to environmental exposure is difficult. For example, depending on the species and environment, stressors can be nutritional [69], osmotic [70] or thermal [71]. In each of these examples, the effect of the combination of insults (pollutant burden and other stressor) resulted in differences in tissue EDC burdens or enhanced susceptibility to the pollutants. Interactions between EDCs and other factors are poorly understood but may be a significant factor in animal health because subtle, underlying physiological disruptions that have no detectable effect in the healthy, unstressed, animal may become important when combined with other influences. Domestic animals can also suffer from such stressors and, in modern production systems, particularly social stressors [72] but the effects of such factors on responses to EDCs has been little investigated in domestic animals at this time.

The future: Since EDCs are not causing widespread, acute, animal health issues, they may seem unimportant. However, it is important to remember that many of their effects are very subtle, and include changes in expression of particular genes, in fetal organ development and in animal reproductive performance and health, none of which are readily detected. Evidence of health effects in humans include increased incidences of testicular abnormalities and reduced fertility [22]. However, while the body of circumstantial evidence indicating a probable role of EDCs in these effects is large and rapidly growing, generally, it is virtually impossible to demonstrate, directly, causal links between EDC exposure and effect.

Setting aside the overarching threat that EDCs may pose to animal and human health and ecosystem sustainability, in practice, effects of EDCs may be of concern because of small effects on the long term health and productivity of domestic animals [39], particularly if they are acting in conjunction with other adverse influences. Thus, subtle effects of low level, environmental exposure to EDCs and effect.

The extent to which animals are exposed to EDCs, and therefore the associated risk, is likely to depend on species and management practice
e.g. with increasing costs of artificial fertiliser, the application to land of processed wastes such as sewage sludge and green waste compost is increasing. Production systems are also moving to the extremes; while low input systems are increasingly favoured in hill and upland areas, this in turn results in more intensive production and increased pesticide use in areas of high quality land and perhaps higher densities of animals with associated social stresses. Each trend has implications for the rate of EDC exposure and for the effects of exposure, as indicated above.

It is easy to focus entirely on the negative consequences associated with the production and use of EDCs but it is important to recognise the need for a more balanced view. Taking the use of analgesics as an example, while they may have disruptive effects in fetal development, this disbenefit must be considered in the light of the enormous potential benefits of their use. Similarly, while pesticides, herbicides, components of plastics and many other everyday products contain EDCs which may pose an insidious threat to animal health and productivity, they also provide massive benefits in terms of food production and human and animal health. Thus, optimising their production and use may not be easy and will certainly require a better understanding of the rates of exposure to, and actions of, these chemicals.

References
Eggshell thinning, chlorinated hydrocarbons, and...
quickly assess the extent of the contamination, and therefore can limit the spreading of the problem and allow the early market release of food, feed and related products once they have been cleared by the fast screening tool. Nevertheless, the introduction of fast bio-analytical tools by various EU member countries and in the World is slow, mostly due to a variety of barriers (acceptance, psychological, competition) well-known to exist for introduction of novel technologies. However, in food crisis situations, affected countries show a high willingness to quick adoption and implementation of fast screening methods, in order to provide for sufficient analytical capacity and to limit the economic damages due to e.g. prolonged export bans. In this presentation the use and application of CALUX as a screening tool is given, as well as its application in several food crisis cases will be presented, including the recent German Dioxin crisis in 2011.

S4 Contamination of livestock due to the operation of a small waste incinerator: a case incident in Skutulsfjörður, Iceland, in 2010

Thorhalur I. Halldorsson1†, Guðjón Atlí Aðalason1†, Rannveig Guicharnaud2, Olafur Dyróymisson3, Sigfúrur Örn Hanssinn3, Kjartan Hreinsson4

1Unit for Nutrition Research, Landspáttú University Hospital, Iceland; 2Faculty of Food Science and Nutrition University of Iceland, Reykjavik, Iceland; 3Innovation Centre Iceland; 11 Reykjavik, Iceland; 4Agricultural University of Iceland, Hvanneyri, Iceland. *The Farmers Association of Iceland, Reykjavik, Iceland; †Icelandic Food and Veterinary Authority (MAST), Iceland.

E-mail: kjartan.hreinsson@mast.is

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Introduction: Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), referred to as dioxins, are formed as unintentional by-products in various industrial processes including waste incineration. Dioxins may also be formed by natural processes like natural fires of vegetated areas but these sources are usually of much less importance than the anthropogenic ones. Other compounds possess dioxin-like properties, notably some polychlorinated biphenyls (PCBs), i.e. the dioxin-like PCBs (DL-PCBs). Based on adverse developmental effects observed in laboratory animals, the tolerable weekly intake of dioxins and dioxin-like PCBs for humans has been estimated to be 14 pg WHO1998-TEQ/kg b.w. [1]. Dioxins and dioxin-like PCBs are also classified as human carcinogens [2] and human exposure to these contaminants has been associated with a number of other adverse health effects [3-5]. The toxicity of dioxins and dioxin-like compounds is quantified in terms of toxic equivalents (TEQ) calculated by way of toxic equivalent factors (TEFs), which rank the different congener’s relative toxicity towards the most toxic dioxin, 2,3,7,8-TCDD, which has a TEF equal to one [6]. There are 210 possible congeners of PCDD/Fs and 209 congeners of PCBs of which seventeen and two respectively. Dioxin persistent compounds with elimination half-life in humans ranging from 1 to >20 years [7]. Dioxins and PCBs accumulate in fat and biomagnify in the food web of aquatic and terrestrial animals. As a result, foods of animal origin usually account for more than 90% of human exposure [8].

Levels of dioxins and PCBs in humans have decreased to less than 20% of peak levels that were observed in the early 1970s [9]. This decrease is likely to reflect the reduction in use of organochlorine compounds and introduction of strict legislation on emissions for example incineration processes [10]. Stipulation of maximum and action limits for dioxins and dioxin-like PCBs in food and feed may also have played some role [11]. Despite considerable emphasis of reducing dioxins and dioxin-like PCBs in food and feed, incidents of accidental food contamination are occasionally reported. In some cases contamination has occurred when contaminated oils or additives are accidentally mixed with animal feed [12-14]. Direct contamination of livestock through intake of contaminated plants and soils appears to be less frequent. This is mainly due to the chemical characteristics of dioxins which are characterized by low vapor pressure, low aqueous solubility and strong adsorption to organic matter leading to limited water leaching or plant uptake [15,16]. Incident of food contamination through uptake of contaminated plants and soils has, however, recently been reported in the vicinity of Naples, Italy, where the suspected source was illegal open burning of waste [17].

In late 2010, elevated levels of dioxins were detected in milk, beef and lamb in Northwestern Iceland. The contamination was localized to a narrow valley, Engidalur, at the bottom of a fjord, Skutulsfjörður. A small municipal waste incinerator was situated in the valley. Since the contamination was discovered, the Icelandic Food and Veterinary Authority has monitored the area and the results of that work are reported in this paper.

Materials and methods: Case setting: In December 2010 an internal control revealed non-compliant levels of dioxin and dioxin-like PCBs in milk from Skutulsfjörður. The sample was a composite sample form the only dairy farm in the fjord, located in a narrow valley called Engidalur. The valley is situated in the bottom of the fjord with high mountains on each side. Calm weather is dominant in the valley. The dairy farm produced approximately 45 tons of milk annually. Additional agricultural production in Engidalur was approximately 2 tons of meat from the dairy farm (beef and sheep) and 4 tons of sheep meat produced by a nearby farm in the valley. The location of Skutulsfjörður in Iceland is shown in Figure 1. The source of the contamination was a small waste incinerator processing roughly 3000 tons waste per year from the local community. The incinerator was operating within a 2km radius of the two farms. An aerial photograph showing the location of the incinerator and the two farms is given in Figure 2. A previous control inspection of the waste incinerator in 2007 had shown dioxin levels in fly ash of 2.1 ng I-TEQ/m³, which is proximately 20 times higher than the maximum limit of 0.1 ng I-TEQ/m³ set by the current EU incinerator directive [18]. The inspection conducted in 2007 was only the inspection carried out since the operation of the incinerator started in 1995. The incinerator had been operating on a dispensation from the EU incinerator directive. Prior to the current incident, the results from the 2007 inspection were neither made publicly available nor had they been reported to the Icelandic Food and Veterinary Authority.

Collection of samples: As the dairy herd was localized at the farm and feed on hay from the valley, one additional composite milk sample and two samples of beef were considered sufficient for examining contamination at the farm. A composite sample of hay harvested from the farm during the summer was also collected. For control, and to examine the spread of the contamination, 2 composite milk samples were collected from nearby fjords. Average values from a previous survey conducted in 2003-2004 were also used for comparison (n=10).

Evaluating contaminant levels in sheep were hampered by several factors. Firstly the sheep from the dairy farm were fed on hay from Engidalur during winter but grazed in a nearby fjord on the other side of the mountains during the summer. Sheep form the other farm in Engidalur received hay during the winter that was harvested outside Engidalur but the herd grazed in the valley during summer. For examining the contaminant levels in sheep a total of 11 meat samples from Engidalur were collected. For control, average values from samples of lambs from the 2003-2004 survey were used (n=5), and an average value for the three control samples of sheep collected in 2001 (n=1). Eight meat samples collected in Engidalur were from animals that had been slaughtered 2-4 months prior to the incident. Following up on the current incident, the Environment Agency of Iceland examined the levels of dioxins in soils in Engidalur and surrounding area [19]. Soil samples were collected after the overlaying grass turf was removed after which a profile down to 5cm depth was collected [20].

Chemical analysis: The samples of milk, meat and hay were analysed at the National Food Institute, Technical University of Denmark. The analytical method has been described elsewhere [21]. In short, the requirements of the commission regulation (EC) No 1883/2006 for official control of dioxins and dioxin-like PCBs were followed [22]. Fat extraction was performed by accelerated solvent extraction on ASE300 (Dionex). Quantification of dioxins and PCBs was made by gas chromatography and detection by high resolution mass spectrometer (GCHRMS, Trace GC ultra and Finnigan MAT97). The GC was equipped with split/splitless injector and DB5MSDQ column (10m pre-column, 60 m, 0.25 mm i.d., film thickness 0.25 μm). Standards were obtained from Cambridge Isotope Laboratories, INC (USA) and Dr. Ehrenstorfer (Germany). The congeners analyzed were the seven and ten chloro substituted PCDDs and PCDFs, respectively; the four non-ortho PCBs (no. 77, 81, 126 and 169); eight mono-ortho PCBs (no 105, 114, 118, 123, 156, 157, 167 and 189); and the six marker PCBs (no 28, 52, 101, 138, 153 and 180). The Environment Agency of Iceland was responsible for the soil samples. Samples were dried at 105 °C according to DIN 38414-S2 and sieved for...
collection of the <2 mm fraction for analysis, which was carried out by Eurofins GfA, Germany.

Results: Overview of the types of samples collected and description of the sample location is given in Table 1. To examine the spread of the contamination, PCDD/Fs, dioxin-like PCBs and 7-marker PCBs in milk from Engidalur were compared with contaminant levels in samples collected in two nearby fjords. For all sample types (milk, beef, sheep (lambs/ewes), and hay), average values from samples collected in 2003-2004 at various locations around Iceland were also used for comparison. The data of 2003-2004 represent background contamination in Iceland.

Both the composite milk samples collected in Engidalur exceeded the EU maximum limits of 3.0 and 6.0 pg WHO-TEQ/fat for PCDD/Fs and the sum of dioxins and dioxin-like PCBs, respectively [11] (Table 2). The two control samples (A and B), taken in nearby fjords, were far below the maximum limits; with levels of PCDD/Fs of around 0.2 pg WHO-TEQ g/fat and the sum of dioxins and dioxin-like PCBs of 0.7 pg WHO-TEQ g/fat. The contaminant levels in the control samples A and B were close to levels previously observed as background levels in Iceland found in the 2003-2004 survey (n=10). The sum of the 7 marker-PCBs in the milk samples were around 5-fold higher in Engidalur compared to the control samples. As expected, elevated levels of PCDD/Fs and dioxin-like PCBs were also observed in hay from Engidalur. Taking analytical precision into account, the observed levels were marginally but not significantly above the maximum levels of 0.75 pg WHO-TEQ/g for PCDD/Fs and 1.25 pg for total WHO-TEQ/g [23]. In comparison, non-detectable levels for most PCDD/Fs-congeners had previously been observed in hay in the 2003-2004 survey.

The levels of PCDD/Fs and total WHO-TEQ in 11 samples of lambs and ewes from Engidalur are shown in Figure 3. For comparison, average values for samples of lambs from the 2003-2004 survey (n=5) and samples collected in 2011 (n=3) are also shown. Lambs A-D were similar to the control samples. These lambs were born in Engidalur but grazed during their lifespan in a nearby fjord prior to slaughtering. Lambs E-G are known to have grazed, at least partly, in the contaminated valley. Ewes providing samples A-B and E grazed most likely in the valley during summer but these animals received hay from outside the valley during winter. Ewe D is believed to have received hay from the valley during winter but grazed outside the valley during summer.

The congener profiles of PCDD/Fs in the milk and hay samples from Engidalur are shown in Table 3. Both absolute (pg/g) and relative concentrations (% of PCDD/Fs WHO-TEQ) are reported. In short, similar profiles of PCDD/Fs are observed in milk and hay from Engidalur and this profile appears to be different from the relative PCDD/Fs levels in hay from the 2003-2004 survey. A detailed comparison is, however, hampered by many non-detects in the control samples. A more detailed comparison of milk and hay samples is, however, possible for the 7 marker PCBs (Table 4) and the dioxin-like PCBs (Table 5). That comparison reveals a different pattern of marker PCBs in hay from Engidalur compared with hay from the 2003-2004 survey.

Discussion: The current study suggests that the operation of a small municipal waste incinerator, not satisfying modern day emission standards, may result in non-compliant levels of dioxins and dioxin-like PCBs in locally produced foods. The incident was limited to a small area and non-compliant levels in milk and beef were observed. Our results do, however, demonstrate the difficulty of evaluating contaminant levels in lambs and ewes, which could migrate freely in and out of the contaminated area.

When evaluating the contamination in Engidalur, the milk samples were considered most reliable. Firstly, contaminant levels in milk are known to be closely correlated with levels in adipose tissue [24] and since the samples were collected from the milk tank at the farm, they should reflect average contaminant levels in the herd. Secondly, the herd was located at the farm only and was predominantly fed on hay harvested in the valley. The hay sample, which was a composite sample from different bales of hay harvested during the summer, showed PCDD/Fs levels that were at least seven times higher than background values (0.85 versus 0.12 pg total WHO-TEQ/g). It is worth noting in this context that the PCDD/Fs levels in the control sample of hay were mostly lower than the upperbound level of 0.12 pg WHO-TEQ/g, as most congeners in the control sample were below limit of quantification. From these results it was concluded that the source of the contamination was due to deposition of contaminated fly ash from the nearby incinerator.

The congener pattern of PCDD/Fs was similar for the milk and hay samples from Engidalur with 2,3,4,7,8-PeCDF accounting for 30% and 34% of PCDD/Fs in hay and milk from Engidalur but the average contribution for the control milk sample was <3.4%. This pattern of approximately 50% of...
Figure 2 (abstract S4) Aerial photograph of Skutulsfjörður. The numbers give the location of the (1) waste incinerator, (2) nearby sheep farm in Engidalur, (3) nearby dairy farm in Engidalur, and (4,5) local town of Ísafjörður.

Table 1 (abstract S4) Description and specification of samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Year</th>
<th>Type</th>
<th>Location</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engidalur</td>
<td>2010-2011</td>
<td>Milk (composite sample), beef, ewes, lamb and hay, soil</td>
<td>Skutulsfjörður, NW Iceland. See Figure 2</td>
<td>Contaminated area. A dairy farm and a small sheep farm located within 2 km radius from the waste incinerator</td>
</tr>
<tr>
<td>Control A</td>
<td>2010-2011</td>
<td>Milk (composite sample), soil/sediment</td>
<td>Álfafjörður, NW Iceland</td>
<td>Nearby fjord on the other side of the mountains, south east from Engidalur (= 10 km direct distance)</td>
</tr>
<tr>
<td>Control B</td>
<td>2010-2011</td>
<td>Milk (composite sample)</td>
<td>Önundarfjörður, NW Iceland</td>
<td>Nearby fjord on the other side of the mountains, south west from Engidalur (= 10 km direct distance)</td>
</tr>
<tr>
<td>Control C</td>
<td>2003-2004</td>
<td>Milk (composite samples), beef, lamb, hay</td>
<td>Different locations in Iceland</td>
<td>Average values from a previous control survey</td>
</tr>
</tbody>
</table>
the total PCDD/Fs contribution from 2,3,4,7,8-PeCDF and 1,2,3,7,8 PeCDD is not fully consistent with the pattern observed in the recent incident in Italy [17] were the source of the contamination was also considered to be burning of waste. In contrast to our setting, the Italian incident was most likely related to open burning of waste while in our case the source can be considered more controlled (although not up to date with modern standards). Studies have also shown that the congener pattern depends of the type of material being burned as well as the temperature [25,26],

Table 2 (abstract S4) Upperbound levels of PCDD/Fs, dioxin-like PCBs and marker PCBs in milk, beef and hay from Engidalur in comparison to control samples

<table>
<thead>
<tr>
<th>Sample no. and type</th>
<th>PCDD/Fs</th>
<th>DL-PCBs</th>
<th>Total TEQ</th>
<th>Sum 7 PCBs</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pg WHO-TEQ g/fat</td>
<td>ng g/fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite milk samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1 Engidalur</td>
<td>3.98</td>
<td>5.33</td>
<td>9.39</td>
<td></td>
<td>Non-compliant</td>
</tr>
<tr>
<td>M2 Engidalur</td>
<td>4.91</td>
<td>8.72</td>
<td>10.24</td>
<td></td>
<td>Non-compliant</td>
</tr>
<tr>
<td>M3 Control A</td>
<td>0.17</td>
<td>0.53</td>
<td>0.33</td>
<td>2.0</td>
<td>Compliant</td>
</tr>
<tr>
<td>M4 Control B</td>
<td>0.22</td>
<td>0.35</td>
<td>0.75</td>
<td>2.4</td>
<td>Compliant</td>
</tr>
<tr>
<td>M5 Control C</td>
<td>0.14</td>
<td>0.40</td>
<td>0.54</td>
<td>4.4</td>
<td>Compliant</td>
</tr>
<tr>
<td>Beef</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1 Engidalur</td>
<td>4.68</td>
<td>7.63</td>
<td>12.31</td>
<td>21.7</td>
<td>Non-compliant</td>
</tr>
<tr>
<td>B2 Engidalur</td>
<td>2.66</td>
<td>2.90</td>
<td>5.56</td>
<td>9.3</td>
<td>Above action level</td>
</tr>
<tr>
<td>B3 Control C</td>
<td>0.18</td>
<td>0.39</td>
<td>0.57</td>
<td>1.85</td>
<td>Above action level</td>
</tr>
<tr>
<td>Composite hay sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1 Engidalur</td>
<td>0.85</td>
<td>0.51</td>
<td>1.36</td>
<td>1.3</td>
<td>Above action level</td>
</tr>
<tr>
<td>H2 Control C</td>
<td>0.11</td>
<td>0.12</td>
<td>0.23</td>
<td>0.9</td>
<td>Above action level</td>
</tr>
</tbody>
</table>

1 PCB number 28, 52, 101, 118, 138, 153 and 180.
2 According the COMMISSION REGULATION (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs and COMMISSION RECOMMENDATION (EC) No 88/2006 on the reduction of the presence of dioxins, furans and PCBs in feedingstuffs and foodstuffs.
3 Average of 10 samples.
4 Average of 4 samples for the PCDD/Fs and 2 samples for the sum 7 PCBs (the 7 marker PCBs were not measured in two samples). Levels in all hay samples are based on 12% moisture content.

Figure 3 (abstract S4) Concentration of PCDD/Fs and total dioxins and dioxin-like PCBs in lamb and ewes from Engidalur 2011. Average values in Icelandic lamb from a previous survey in 2003-2004 (n=5) and control samples taken in 2011 (n=3) are also presented as a comparison. Lamb A-D are lambs from Engidalur that are known to have grazed outside Engidalur prior to slaughtering. Lambs E-G are known to have grazed in Engidalur. The four ewe samples were from Engidalur with ewes A-B and E grazing in the valley during summer but receiving hay from outside the valley during winter. Ewe D received hay from the valley during winter but grazed outside the valley during summer.
which may explain the unique pattern that appears to occur in each food contamination incident [12-14,17].

Unlike the case for the PCDD/Fs, the marker PCBs (Table 4) show a marked difference in relative distribution between the hay and the milk, where the contribution of the lighter PCBs (PCBs #52, 52 and 101) is much higher in the hay than in milk indicating different uptake and/or elimination routes of light versus heavy PCBs in the milking cows. This is supported by the fact that the relative distribution of marker PCBs is similar in milk of both control sample and the samples from Engidalur in spite of the fact that the marker PCBs in the control hay-samples have a greater contribution of the lighter PCBs than in Engidalur. The greater contribution of lighter PCBs in the control hay indicates long-range atmospheric transport in the background samples while a more local source seems to be affecting the sample in Engidalur. It is noticeable that the levels of marker PCBs in the hay are somewhat higher in the control samples than in the samples from Engidalur, most likely reflecting both temporal and spatial trends.

The congener pattern of dioxin-like PCBs is similar for the milk and hay samples from Engidalur with contribution of PCB-126 dominating with 86 and 81% of the PCBs' WHO-TEQ, respectively (Table 5). The relative distribution of dioxin-like PCBs in Engidalur differs from the background in mostly higher contribution from PCB-126 in Engidalur, i.e. by 86% and 41% for Engidalur and control, respectively. The concentration differences in dioxin-like PCBs in Engidalur and control in terms of TEQ concerns about 5. Unlike the relative distribution of non- and mono-ortho PCBs in hay, the relative distribution of dioxin-like PCBs in milk from Engidalur and milk from the background is fairly similar where the main contribution to the WHO-TEQ in both is from PCB-126 or 80%. The concentration difference in milk for the dioxin-like PCBs in terms of TEQ is, however, large or about 11-fold.

Dioxins and PCBs have high affinity to organic matters in soils and sediments, compartments that are regarded as major sinks and in which slow degradation takes place. Transfer of dioxins from soil to plants is considered very limited but grazing animals and humans may be

Table 3(abstract S4) Comparison between different PCDD- and PCDF-congeners in milk and hay form Engidalur with background levels. Both absolute concentrations and the relative contributions of TEQ of each congener are presented.

<table>
<thead>
<tr>
<th>Hay (pg/g)</th>
<th>Milk (pg/g fat)</th>
<th>Hay (% of PCDD/Fs WHO-TEQ)</th>
<th>Milk (% of PCDD/Fs WHO-TEQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8 TCDF</td>
<td>&lt;0.05</td>
<td>0.13</td>
<td>0.29</td>
</tr>
<tr>
<td>1,2,3,7,8 PeCDD</td>
<td>0.41</td>
<td>&lt;0.03</td>
<td>0.17</td>
</tr>
<tr>
<td>2,3,4,7,8 PeCDF</td>
<td>0.15</td>
<td>&lt;0.03</td>
<td>3.18</td>
</tr>
<tr>
<td>1,2,3,4,7,8 HxCDF</td>
<td>0.56</td>
<td>&lt;0.03</td>
<td>1.72</td>
</tr>
<tr>
<td>1,2,3,6,7,8 HxCDF</td>
<td>0.75</td>
<td>&lt;0.03</td>
<td>2.14</td>
</tr>
<tr>
<td>2,3,4,6,7,8 HxCDF</td>
<td>0.89</td>
<td>&lt;0.03</td>
<td>2.78</td>
</tr>
<tr>
<td>1,2,3,8,9 HxCDF</td>
<td>0.01</td>
<td>&lt;0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8 HpCDF</td>
<td>2.60</td>
<td>0.14</td>
<td>0.74</td>
</tr>
<tr>
<td>1,2,3,4,7,8,9 HpCDF</td>
<td>0.24</td>
<td>&lt;0.04</td>
<td>0.15</td>
</tr>
<tr>
<td>OCDF</td>
<td>0.99</td>
<td>0.22</td>
<td>0.40</td>
</tr>
<tr>
<td>2,3,7,8 TCDD</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
<td>0.42</td>
</tr>
<tr>
<td>1,2,3,7,8 PeCDD</td>
<td>0.19</td>
<td>&lt;0.03</td>
<td>1.48</td>
</tr>
<tr>
<td>1,2,3,4,7,8 HxCDD</td>
<td>0.15</td>
<td>&lt;0.03</td>
<td>0.61</td>
</tr>
<tr>
<td>1,2,3,6,7,8 HxCDD</td>
<td>0.28</td>
<td>&lt;0.03</td>
<td>1.20</td>
</tr>
<tr>
<td>1,2,3,7,8,9 HxCDF</td>
<td>0.19</td>
<td>&lt;0.03</td>
<td>0.47</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8 HpCDD</td>
<td>1.70</td>
<td>0.35</td>
<td>1.07</td>
</tr>
<tr>
<td>OCDD</td>
<td>2.0</td>
<td>1.35</td>
<td>5.96</td>
</tr>
</tbody>
</table>

1 The results are based on hay with 12% moisture content.
2 Average of 4 samples.
3 Average of 10 samples.

Table 4(abstract S4) Comparison between the different 7 marker PCBs in milk and hay form Engidalur with background levels. Both absolute concentration and relative concentrations for each congener are reported.

<table>
<thead>
<tr>
<th>Hay (ng)</th>
<th>Milk (ng/g fat)</th>
<th>Hay (% of sum 7 PCBs)</th>
<th>Milk (% of sum 7 PCBs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>0.11</td>
<td>1.20</td>
<td>0.16</td>
</tr>
<tr>
<td>52</td>
<td>0.18</td>
<td>0.76</td>
<td>0.10</td>
</tr>
<tr>
<td>101</td>
<td>0.33</td>
<td>0.93</td>
<td>0.22</td>
</tr>
<tr>
<td>118</td>
<td>0.31</td>
<td>0.30</td>
<td>4.36</td>
</tr>
<tr>
<td>138</td>
<td>0.21</td>
<td>0.34</td>
<td>2.36</td>
</tr>
<tr>
<td>153</td>
<td>0.19</td>
<td>0.34</td>
<td>2.32</td>
</tr>
<tr>
<td>180</td>
<td>0.03</td>
<td>0.06</td>
<td>0.33</td>
</tr>
</tbody>
</table>

1 PCB number 28, 52, 101, 118, 138, 153 and 180.
2 Based on hay with 12% moisture content.
3 Average of 2 samples.
4 Average of 10 samples.
The contamination was discovered in Engidalur, collection of milk and meat in Engidalur of 45 and 6 tons, respectively, the dioxin incident reported in this paper cannot be considered as significant in terms of the amount of contaminated foods distributed or the number of consumers affected. Location of the ad hoc working group, all animals used for food production from the two farms in question were culled and all potentially contaminated feed has been disposed of. This decision is in slight contrast to the recent incident in Italy were non-compliant herds from around 100 farms were monitored at 45 day intervals until compliant levels in milk were observed [17]. In the current case the number of potentially contaminated animals was much smaller and this incident was not confined to dairy cows only.

Using similar approach as in Italy would have been considerably more expensive and potentially less effective than culling the animals. Following up on this incident and in the light of divergent results on hay and soil samples, a grazing experiment is currently being conducted to evaluate whether the uptake of dioxins and dioxin-like PCBs still occurs from plants or from the surface layer of the soils in Engidalur. The results of that experiment will be used for determining next steps with respect to the agricultural activities in the area. The incinerator in Engidalur was new when it first started its operation in 1995 and can therefore not be considered as a typical “old” incinerator often associated with elevated dioxins emissions [31]. The incinerator did, however, receive dispensation from the EU regulations on maximum limit of dioxins in fly ash of 0.1 ng I-TEQ/m³. The argument for seeking dispensation was on one hand the relatively high cost of meeting this requirement for a unit with such a small throughput (=3000 tons waste/year) and on the other hand it was considered unlikely that such a small incinerator could have a significant impact on the local surroundings. That decision was not reassessed after the inspection of the incinerator in 2007 which showed elevated emission levels. Based on that single measurement, the annual emission of dioxins from the incinerator was estimated to be 0.087 g I-TEQ. The current incident does therefore support the EU regulation on dioxins emissions from incinerators and demonstrates that dispensation from the regulation may result in contamination of the environment. Dispensation should at least be followed up by a continuous monitoring and inspection of the nearby surroundings to ensure its rationale.

Summary: Although limited in scope, the current incident clearly demonstrates that operation of a small waste incinerator that is non-compliant with the current EU legislation may result in elevated levels of dioxins in foods in the nearby surroundings. This incident also demonstrates the difficulty of tracing contaminant sources by migratory animals such as sheep that can freely move in and out of the contaminated area. With respect to future monitoring programs conducted by the Icelandic Food and Veterinary Authority, the incident highlights the need of good flow of information between different inspection authorities; and the importance of targeted monitoring of food production close to potential sources of contamination, even though the sources may be relatively small in scale.

Table 5 (abstract S4) Comparison between different dioxin-like PCB congeners in milk and hay form Engidalur with background levels. Both absolute concentrations and the relative contributions of TEQ of each congener are presented

<table>
<thead>
<tr>
<th>PCB</th>
<th>Hay (pg/g)</th>
<th>Milk (pg/g fat)</th>
<th>Hay (% of PCB WHO-TEQ)</th>
<th>Milk (% of PCB WHO-TEQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Engidalur</td>
<td>Engidalur Control C²</td>
<td>Engidalur</td>
<td>Engidalur Control C³</td>
</tr>
<tr>
<td>PCDD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCDD-77</td>
<td>28</td>
<td>68</td>
<td>&lt;3</td>
<td>0.55</td>
</tr>
<tr>
<td>PCDD-81</td>
<td>1.8</td>
<td>3.0</td>
<td>&lt;0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>PCDD-126</td>
<td>4.4</td>
<td>35.5</td>
<td>2.7</td>
<td>86.1</td>
</tr>
<tr>
<td>PCDD-169</td>
<td>0.77</td>
<td>7.5</td>
<td>0.7</td>
<td>1.5</td>
</tr>
<tr>
<td>PCDF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCDF-105</td>
<td>130</td>
<td>1071</td>
<td>146</td>
<td>2.5</td>
</tr>
<tr>
<td>PCDF-114</td>
<td>7</td>
<td>118</td>
<td>17</td>
<td>0.69</td>
</tr>
<tr>
<td>PCDF-118</td>
<td>310</td>
<td>4358</td>
<td>10</td>
<td>6.1</td>
</tr>
<tr>
<td>PCDF-123</td>
<td>&lt;5</td>
<td>80</td>
<td>&lt;11</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>PCDF-156</td>
<td>22</td>
<td>231</td>
<td>67</td>
<td>2.2</td>
</tr>
<tr>
<td>PCDF-157</td>
<td>3</td>
<td>56</td>
<td>19</td>
<td>0.29</td>
</tr>
<tr>
<td>PCDF-167</td>
<td>5</td>
<td>107</td>
<td>55</td>
<td>0.01</td>
</tr>
<tr>
<td>PCDF-189</td>
<td>1</td>
<td>17</td>
<td>&lt;10</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1 The results are based on hay with 12% moisture content.
2 Average of 4 samples.
3 Average of 10 samples.
Competing interests: The authors declare that they have no competing interests.

Authors’ contributions: KH and SOH designed the experiment and collected the samples. TIH, GAA, OD, and RG provided expert advice during the experiment and with respect to interpretation of the results. TIH and GAA drafted the manuscript with input and critical revisions from KH, SOH, OD and RG.

Acknowledgements: The authors acknowledge the contribution of late Dr. Olofur Guðmundsson for his work on collecting information on dioxins and PCBs in hay samples in the 2003-2004 survey. The Agricultural Productivity Fund in Iceland acknowledged for their financial support in carrying out the 2003-2004 surveillance project. We would also like to acknowledge the help of District Veterinarian Sigurður Ingó Sigurjónsson and the contribution of the farmers Steingrimur Jónsson and Kristján Ólafsson for their assistance with sample collection.

References

Mycotoxins are toxic secondary fungal metabolites. A large variety of mycotoxins have been described, but only a minority of them occur regularly in feed and food items under normal conditions and pose a risk to feed and food safety.

The important moulds producing toxins during storage belong to the genera of 

Pennylium, or Aspergillus. The most important storage toxins are probably ochratoxin A and aflatoxins. The growth of moulds and toxin production after harvest are dependent on temperature and humidity and, consequently, the most effective mitigation strategy is to maintain the commodities dry and at a low temperature during storage. Aflatoxin is normally not produced in the Norwegian climate and the occurrence is restricted to imported feed and food. Ochratoxin A is produced by 

Pennylium spp and Aspergillus spp. Occurrence of ochratoxin A in selected commodities is included in the national food and feed surveillance programs. Only low concentrations of ochratoxin A have been found in compound feed as well as in feed ingredients [1-5]. It is concluded that the procedures for handling of feed seem to be sufficient to limit the production of ochratoxin A.

Elevated levels of ochratoxin A are occasionally reported from dried food items, including dried fruits and coffee beans. The fungi producing aflatoxin are generally restricted to a tropical climate. Consequently, aflatoxins in Norwegian feed and food are mainly limited to imported feed, food and ingredients. The main aflatoxin sources in feed have been maize and maize-derived feed ingredients, but the levels in compound feed have been low [1-5]. Aflatoxins do not constitute a high risk to animal health nor to human health through transfer to humans through consumption of animal-derived food products in Norway. The toxin occurs in imported dried feed items such as dried figs and nuts, but the results from the present control regime shows that the risk to human health due to consumption of aflatoxin-contaminated foods is limited.

Penitrems: Penitrems are neurotoxic mycotoxins produced by 

Pennylium spp. Penitrems have generally been reported from visibly damaged commodities, and reported intoxications from humans are limited. Dogs, however, may ingest mouldy commodities such as mouldy food or feed, food waste or apples lying on the ground. Ingestions of such items have been reported to result in severe intoxications with a variety of clinical symptoms characteristic for neurotoxic poisoning, including shivering, tremors, and tachycardia [6,7].

Trichotecenes: Trichotecenes are a large family of related mycotoxins, but only deoxynivalenol (DON), and T-2 and HT-2 toxins are frequently found in Norwegian grains. Trichotecenes have been analysed in grains sampled at delivery for many years. The levels of DON in Norwegian grains are low, but the toxins have also been analysed in processed feed. Other trichotecenes such as nivalenol (NIV) are also found in Norwegian grains, but both frequency of positive samples and the concentrations reported are generally low [1-5]. The levels are, with few exceptions, below the maximum limits for feed ingredients. According to EFSA, DON in feed may affect feed intake of pigs at levels from 0.35 – 0.9 mg/kg feed [8]. The levels of DON in pig feed are therefore close to those that may have a negative impact on feed intake. Other species are less sensitive, and no effects on other species are expected at the current levels in feed. The apparent increasing trend of DON in Norwegian grains is also of concern.

Ergot: Ergot refers to fungi of the genus 

Claviceps, where 

C. purpurea is the most prominent. Presumptive intoxications of moose and roe deer have been reported from different parts of Norway. Three moose were found dead, while the remaining moose and the roe deer were all suffering from typical signs of ergotism, like convulsive and gangrenous symptoms [9]. Fungal sclerotia have later been collected from grass in the pasture in the area and ergotamine and analogues have been identified and described [10]. These toxins are not analysed for in processed feed. The fungus is common in rye, but the toxin-containing sclerotia are removed during processing at the mills. Traces have been reported from wheat and barley in other countries and may be present even in Norway. The levels have not been regarded as any significant risk to animal health or welfare.

Conclusions: Trichothecenes and DON in particular, are the main trichothecenes of concern in processed feed. DON occurs in processed feed in concentrations close to levels found to reduce the feed intake and feed conversion in pigs. The neurotoxic mycotoxin penitrem A is of concern for animals consuming moldy feed, and in particular for dogs ingesting food waste and roasted and molded apples. Grazing wild-life, such as moose and roe deer, may be intoxicated by ergot.

References
7. European Food Safety Authority: Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to deoxynivalenol (DON) as undesirable substance in animal feed. The EFSA J 2004, 74-112.
prospective research, in this case based on birth cohorts. Given the advantages of conducting such research in the Faroe Islands, we have therefore generated five birth cohorts. In addition, we have used available records on whaling during the past century to clarify prenatal methylmercury exposure of elderly people on the basis of availability.

**Cohort 1:** A cohort of 1022 singleton births was assembled in the Faroe Islands during a 21-month period of 1986-1987. The range of mercury concentrations in cord blood and maternal hair was about 1000-fold. Frequent whale meat dinners during pregnancy and, to a much lesser degree, frequent consumption of fish, and increased parity or age were associated with high mercury concentrations in cord blood and maternal hair. The levels were slightly lower if the mother had ingested alcoholic beverages (which happened only occasionally in this group). Mercury in cord blood correlated moderately with blood-selenium. Lead in cord blood was low (median, 82 nmol/l), particularly when the mothers had frequently had fish for dinner and abstained from smoking. Because the effects of fetal childhood exposure to methylmercury are persistent, detailed examination of children with prenatal exposure to this neurotoxicant would be appropriate at school age. At this time, they have developed sufficiently to perform a wide variety of neurobehavioral tests, and they are capable of cooperating for most functional tasks. The first detailed examination took place at age 7 years, i.e., just before school attendance (April 1993 and February 1994) and was aimed at the oldest children of the cohort, at the same time in 1994. Children currently residing in Denmark were examined in 1994. A total of 917 of the surviving children (90.3%) completed the examinations. Because of a slightly lower participation rate of children from the capital of Torshavn, the prenatal mercury exposure levels of the children examined was significantly higher [geometric mean cord-blood mercury concentration, 22.8 µg/l (114 nmol/l)] than that of those who did not participate [17.9 µg/l (89 nmol/l)]. Because no other selection bias was apparent, the small attrition would be unlikely to affect a relationship between mercury exposure and neurobehavioral function. Most of the children were examined at the National Hospital in Torshavn, the capital of the Faroe Islands. All transportation costs were refunded. Four children were examined during the morning and four during the afternoon at five examination stations, with each station taking up to 60 minutes. Past medical history, current health status and social factors were recorded on a self-administered form by the parent accompanying the child (usually the mother). The physical examination included a functional neurological examination with emphasis on motor coordination and perceptual-motor performance. Visual acuity was determined by Snellen’s board and contrast sensitivity by the Functional Acuity Contrast Test. Otoscopy and tympanometry were supplemented by audiometry. Main emphasis was placed on detailed neurophysiological and neuropsychological tests that had been selected on the basis of a range of considerations. Tests were chosen to include tasks that would be affected by the neuropathological abnormalities seen in congenital methylmercury poisoning and the functional deficits seen in children with early-life exposure to neurotoxicants. The tests also had to be acceptable to the children and their parents, viz. painless, not too time-consuming, and appropriate for 7-year-old Faroese children who had not yet begun school. Tests that were likely to provide a high statistical sensitivity, i.e., with a wide range of scores possible without floor or ceiling effects, and acceptable test-retest reliability, were preferred. In addition, test versions standardized in Scandinavian countries were favored. The second examinations were completed at age 14 years. Again, the participation rate was very high, almost 90%. The overall approach was very similar to the one previously applied, though the clinical tests were adjusted to be appropriate for the teenage participants. Likewise, comprehensive examinations were carried out at age 23 years. As many cohort members had moved to Denmark for education or employment, a clinic was also established in Copenhagen. Educational achievement is being evaluated using the results from the standardized tests at completion of the 9th grade and the status at age 22 years.

**Cohort 2:** The findings from Cohort 1 suggested that exposure assessment should encompass several lipophilic pollutants in addition to methylmercury. As a follow-up, Cohort 2 was therefore established during a 12-month period in 1994-1995 and included 182 singleton term births from consecutive births at the National Hospital in Torshavn, Faroe Islands. Maternal residence was required in the central and northwestern region of the primary catchment area, i.e., away from the capital area of Torshavn. About one-third of the Faroese population resides in this area, where the mercury exposure was expected to vary the most. A total 64% of all births were included, incomplete sampling being mainly due to logistic problems in the busy ward. The overall participation rate was slightly below the one obtained in Cohort 1, but the average birth weight was almost the same in the two cohorts and similar to the Faroese average. Relevant obstetric data were obtained by standardized procedures and supplemented by a brief nutrition questionnaire. These children were first examined by the Neurological Optimality Score at age two weeks (adjusted for gestational age), and then again at 7 months of age. Subsequent examinations were at age 18 months and then at 2-month intervals up to age 66 months. At 42 months, a comprehensive medical examination with the Neurological Optimality Score was included. For comparison with Cohort 1, detailed neurobehavioral tests were carried out at age 7 years. A repeat examination was then completed at age 10 years. The complete profile of neurobehavioral development is currently being analyzed. This cohort also participated in a study of serum antibody concentrations as a measure of the effects of routine childhood immunizations.

**Cohort 3:** New insight into health risks caused by environmental pollutants and changing exposure patterns in the Faroes lead to the formation of Cohort 3 from consecutive births in Torshavn between April 1998 and 29 February, 2000. Because of dietary recommendations from the Faroese health authorities, methylmercury exposures had now decreased thus allowing better characterization of possible effects of PCBs and other lipophilic contaminants. The main part of Cohort 3 consists of 547 children. Inclusion criteria required appropriate biological specimens for exposure biomarker determination and a valid examination by the pediatrician at two weeks of age. The children included represent approximately 60% of all pregnancies. Most of the attrition was caused by work schedules in the busy ward or scheduling problems. Cohort members have a slightly greater birth weight and slightly older mothers with a somewhat greater parity as compared to non-responders. On the other hand, the child’s sex and Apgar score are similar in participants and non-participants. In regard to parity, maternal age, smoking and alcohol consumption (very limited), Cohort 3 is quite similar to the two previously generated cohorts. Serum was again collected from the mother at the last antenatal examination (34th week of pregnancy). Other samples collected from the mother-child pairs include cord blood and serum, maternal hair at parturition, and milk on days 3-5 (before mother and child were released) and at two weeks. Nutritional habits were recorded by questionnaire (number of whale meat dinners per month during pregnancy and before pregnancy; number of fish dinners per week; ingestion of blubber with whale meat or fish). A subset of 150 mothers also filled in a detailed food frequency questionnaire. A subgroup of Cohort children was examined with regard to immunological parameters at ages 11 and 18 months. The first comprehensive medical examination was carried out just before the booster vaccination at age 5 years, with subsequent follow-up blood sample one month after vaccination. The children were again examined at age 7 years, with a main focus on immunological parameters. A pre-puberty examination is planned for age 12 years to follow the long-term implications of the immunotoxicity changes already documented.

**Cohort 4:** Subsequent to the decision, in August 1998, by Faroese health authorities to recommend that women should reduce their intake of pilot whale meat and blubber – in order to protect the foetus against adverse effects from food contaminants – we carried out a study to determine the effects of this recommendation. The data collection took place during 12 months from October 2000, and 148 women (49.7% of all eligible) completed the full protocol. Blood samples from the 37th week of pregnancy were analyzed for heavy metals and organochlorine compounds. In addition, we used a 24-hour recall as well as a food diary, and also a food frequency questionnaire for the past 12 months to take in account seasonal differences. The women were interviewed at home when they were about 28, 33 and 38 weeks pregnant. To estimate the portion of nutritional intake provided by pilot whale meat and blubber, we carried out a study to determine the effects of this recommendation. The data collection took place during 12 months from October 2000, and 148 women (49.7% of all eligible) completed the full protocol. Blood samples from the 37th week of pregnancy were analyzed for heavy metals and organochlorine compounds. In addition, we used a 24-hour recall as well as a food diary, and also a food frequency questionnaire for the past 12 months to take in account seasonal differences. The women were interviewed at home when they were about 28, 33 and 38 weeks pregnant. To estimate the portion of nutritional intake provided by pilot whale meat and blubber, we carried out a study to determine the effects of this recommendation.
In this study, the association between persistent environmental pollutants and the risk of developing Parkinson's disease (PD) was investigated. The study aimed to assess the association between previous exposure to marine food contaminants and the development of PD, with a focus on immunological parameters.

The most recent cohort was born during an 18-month period between October 2007 and April 2009. The total of mother-child pairs were examined at age 18 months, with more than 3 out of 4 contributing a blood sample. Follow-up will include a 42-month examination and detailed clinical examinations at 5 years of age, with emphasis on immunological parameters.

Septuagenarians: To complement the birth cohorts, studies are also being carried out to examine the health status of elderly Faroese residents in regard to their lifetime exposure to marine pollutants. In this population, where the contaminants mainly originate from pilot whale blubber and meat, past exposures can be estimated from dietary questionnaires. Persistent substances can also be measured in serum. A total of 343 maternal and 206 of the paternal grandparents participated. The children have been examined at age 18 months, with more than 3 out of 4 contributing a blood sample. Follow-up will include a 42-month examination and detailed clinical examinations at 5 years of age, with emphasis on immunological parameters.

The results of the above-mentioned studies together with the findings of other studies, including those that investigate the associations between exposure to persistent environmental pollutants and the risk of developing PD, suggest a decrease in the antibody concentration by about 20% for each doubling in PCB exposure. At age 5 years, the odds of an antidiphtheria antibody concentration below a clinically protective level of 0.1 IU/L increased by about 30% for a doubling in PCB in milk and 18-month serum. In conclusion, developmental PCB exposure was associated with immunotoxic effects on serum concentrations of specific antibodies against diphtheria and tetanus vaccines. The immune system development during the first years of life appears to be particularly vulnerable to this exposure [7-9].

Marine food pollutants as a risk factor for hypoinsulinemia and type 2 diabetes: Clinical examinations of 713 Faroese residents aged 70–74 years (64% of eligible population) included fasting plasma concentrations of glucose and insulin, and glycosylated hemoglobin. Lifetime exposure to persistent environmental chemicals from pilot whale and other traditional food was estimated from a dietary questionnaire and related contaminants suggested slightly increased ORs, although only b-hexachlorocyclohexane (b-HCH) was statistically significant. Increased intake of whale meat and blubber in adult life was significantly associated with PD, thus suggesting a positive association between previous exposure to marine food contaminants and development of PD [10].

The risk of hypertension and arteriosclerosis of the carotid arteries is increased in adults who have an increased exposure to mercury [12]: We examined 42 male members (aged 30-70 years) of the Faroese whaling society to assess possible adverse effects within a wide range of methylmercury exposures from consumption of pilot whale meat. Exposures were assessed from mercury analysis of toenails, scalp hair, and whole blood, including a hair sample collected five years previously. Physiological measures included heart rate variability (HRV), blood pressures, common carotid intima-media thickness (IMT), and brainstem auditory evoked potentials (BAEPs). Structural equation analysis was carried out with independent exposure variables and outcome groupings, with adjustment for confounders, to determine the overall effect of mercury exposure on outcomes. Because of high correlations among related measures, the outcome groups consisted of the HRV parameters, diastolic blood pressure and heart rate, and BAEP results. The predictive validity of individual exposure biomarkers and a latent exposure variable was compared by multiple regression analysis. The results support the notion that methylmercury exposure promotes the development of cardiovascular disease, as expression by increased blood pressures and IMT [11].
Perspectives: Recent risk assessments add perspective to the current limits for mercury concentration in fish. Because of beneficial nutrients, two seafood dinners per week is generally recommended as part of a varied diet. Two dinners would represent up to about 500 g of seafood. The reference dose determined by the U.S.EPA limit indicates that an adult (weight 70 kg) should not exceed a weekly mercury intake of 50 µg. This means that the seafood should contain an average mercury concentration of no more than 0.14 µg/g. However, pilot whale meat contains in average 20 times as much [13].

According to the precautionary principle expressed in the Faroese Statement the medical authorities in the Faroe Islands have recommended that the government that pilot whale be considered unsafe for human consumption [14]. An allowance of a few grams per day in order to remain below intake levels considered safe is simply not realistic. It is ironic the this remote archipelago, which is not responsible for any significant mercury pollution, must now give up a traditional food source, which has contributed energy and essential nutrients to the population for many centuries.

References
permits hunting all year round in unlimited numbers [17], which facilitates sampling.

**Practical aspects of using the mink as a sentinel species:** Basu et al. (2007) [8] recommend sampling of live animals, as repeated measurements and radiotelemetry are possible. Such study designs may be beneficial to meet the objectives of some studies, but are not suitable for large scale screening. In Sweden, live traps have to be attended two times per day, morning and evening [18], which is demanding in terms of labour; the number of collected samples would be quite limited because of the economic cost. Naturally, only a limited geographical area would be covered.

In order to sample a large amount of carcasses from large geographical areas, or from distant areas, it is advisable to turn to local hunters. In our experience, trappers are interested and prone to participate in sampling of mink. In Sweden, it is most common to use lethal traps or to hunt by dog and shotgun. The relatively small body of the mink, males weighing approximately 1100 g and females 620 g [19], makes the carcasses easy to handle and transport. In the sampling we are performing, we have chosen to work with frozen carcasses. The reason for this is that it is considerably easier for the hunter to save mink carcasses continuously and send all mink at the same time. Keeping the carcasses frozen also facilitates planning of work for the person doing the sample fresh carcasses. This would demand a lot more work from the hunter and would most probably result in many carcasses being more or less rotten, as transportation often takes at least a day or two. It would also demand that the person doing the necropsies is at constant standby. Collecting frozen carcasses has been successful in our project as we have sampled over 500 mink during the past five years. The major drawback of freezing the animals is that it limits the possibilities of doing sophisticated histology, as the tissue suffers from artefacts due to disruption of cell membranes by freezing and thawing. Even so, we have been successful in measuring the diameter of the seminiferous tubuli in many of the mink testicles [20]. We were also able to evaluate sperm morphology on samples taken from the epididymides. The sperm had damaged acrosomes because of freezing, but besides this it was possible to record all common abnormalities.

**Variation in data from environmental monitoring:** Data from field studies often show a lot of variation in both biological variables and body burden of contaminants. This large variation is influenced by intrinsic and extrinsic factors. Hence, it is critical to identify these factors and to adjust for them when analyzing data. This will increase the precision of exposure and effect data and will facilitate identification of subgroups within populations that are at the highest risk of adverse health effects due to chemical exposure.

Variations in concentrations and patterns of contaminants in wildlife are not only due to temporal and spatial variation, but also biological factors such as for example reproductive stage, age, sex, habitat use, migration and diet [21]. Metabolism is also an important factor, as it includes events such as growth, hibernation and lactation. All these biological and physiological factors may correlate with each other. For example, seasonal variation in contaminant concentrations in a species may be contributed to metabolism (for example lipids), diet, migration and reproduction. Variation in physiological data from field studies could be attributed to differences in reproductive stage, age, sex, body condition etc. Variation could also derive from pathology, due to xenobiotics, infections etc.

**Own studies using the wild mink as a sentinel:** Our sampling design results in collection of both male and female mink from different areas and of different ages all year round. Multiple regression analysis was chosen to characterize which factors that influence physiological traits and exposure levels. In our first study, season and age had significant influence on many of the selected reproductive traits in male mink, but no significant effect from nutritional status was found [20]. Data on brominated flame retardants (BDEs) showed that among many factors studied, nutritional status was a factor that significantly influenced the concentration (p=0.0015) [in manuscript]. By including these influencing factors into a multiple regression model, preliminary data showed a significant positive correlation (p=0.02) between ΣBDE concentration and testicle weight [22].

**Conclusions:** The mink is a good sentinel species, both based on its biological characteristics and for practical reasons. With the aim to sample large numbers of mink over time, from both large and small geographical areas, we successfully used local hunters and handled frozen carcasses. Factors such as age, season and nutritional status must be taken into account when monitoring effects on the reproductive system in the wild mink.

**Competing interests:** The authors declare that they have no competing interests.

**Authors’ contributions:** Please see sample text in the instructions for authors.

SP wrote most of the manuscript. BB, BMB and HK commented on the manuscript. UM planned the manuscript together with SP and contributed to writing the manuscript. All authors read and approved the final manuscript.

**References:**


S10

Trace elements and organic contaminants in tissues of minke whale (Balaenoptera acutorostrata) and its feed from Icelandic waters

Guðbjörg Atlí Auðunnsdóttir1,2, Gíli A Vikingsson1
1Innovation Center Iceland, Department of Analytical Chemistry, Keldaholti; 2Marine Research Institute Iceland, Department of Whale Research, Skulagata 4, 101 Reykjavik, Iceland

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In a research project by Iceland under the auspice of the Marine Research Institute Iceland in 2003-2007, 200 minke whales (Balaenoptera acutorostrata) were caught in Icelandic waters. Within this research effort of Iceland, a comprehensive research activities were devoted to analysis of trace elements and organic contaminants in both different tissues of the minke whale and its possible prey species as revealed by stomach content. The scientific questions asked by these studies were the following:

1. What are the characteristics of the Icelandic minke whale stock as regards environmental contaminants compared to other stocks of minke whale worldwide?
2. Can inorganic and organic contaminants provide answers regarding the prey species of the minke whale?
3. Is it possible to estimate the feed consumption of the minke whale by way of contaminants in its tissues and its prey species?
4. Do biopsies provide information on the level and behaviour of contaminants in the organs of the minke whale?
5. Are the levels of environmental contaminants in tissues of minke whale detrimental to the health of the animals?
6. Do the levels of contaminants restrict the human consumption of minke whale products?

For this purpose the trace elements Hg, Cd, Zn, Cu, Pb, Se, As, Ni, Cr, and Mn were analysed in skin, muscle, liver, kidneys, ovaries and testes of 25 minke whale samples selected with respect to size, sex, and location around Iceland. PCBs, DDTs, HCB, HCHs, toxaphenes, chlordane, dieldrin and PBDEs were analysed in blubber (biopsy and cross section), muscle, and liver while dioxins and dioxinlike PCBs were analysed in the ventral grooves of five animals. All these contaminants were also analysed in 50 samples of probable prey species: whole animals of cod, haddock, pollock, herring, capelin, sandeel, and krill.

The results are described and discussed with respect to the scientific objectives above with due consideration of the biological factors that may affect the levels, e.g. age, sex, nutritional status and trophic level (δ15N and δ13C).

S11

Persistent organic pollutants in Finnish reindeer (Rangifer tarandus tarandus) and moose (Alces alces)

Anniina Suutari1,5, Anja Hallikainen3, Päivi Ruokojärvi1, Hannu Kiviranta3, Mauri Niiminen3, Sauli Laaksonen1
1University of Oulu, Department of Biology, P.O. Box 3000, 90014 Oulu, Finland; 2Finnish Food Safety Authority Evira, Risk Assessment Research Unit, Mustalankatu 3, 00790, Helsinki, Finland; 3National Institute for Health and Welfare, Department of Environmental Health, P.O. Box 95, 70701, Kuopio, Finland; 4Finnish Game and Fisheries Research Institute, Reindeer Research Station, Toivioniemi 246, 99100 Kaamanen, Finland; 5University of Helsinki, P. O. Box 33, 00014, Helsinki, Finland

E-mail: anniina.suutari@oulu.fi
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Background: Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) are environmentally stable and toxic compounds, listed in Stockholm Convention [1] created to restrict and ultimately eliminate the production, use, release, and storage of Persistent Organic Pollutants (POPs), and observed to exist globally in terrestrial and aquatic biota [2-4]. PCDD/Fs enter the environment solely as unintentional by-products from industrial and thermal processes, while DL-PCBs are merely intentionally produced chemicals that are released due to inappropriate disposal practices, accidents and leakages from industrial facilities [2,5]. As lipophilic substances, PCDD/Fs and PCBs absorb passively from the gastrointestinal tract, enter the circulation and distribute to high lipid tissues such as white adipose. Metabolism and excretion of these compounds are slow leading to accumulation of these substances in the organism. Metabolism or biotransformation can also create reactive intermediates that may cause tissue damage as a consequence of binding to proteins (e.g. transtatheyn), lipids and nucleic acids [2,6,7]. PCDD/Fs and DL-PCBs are able to cause an array of adverse health effects, like cancer, damage to the central and peripheral nervous systems, reproductive and developmental disorders, and disruption of the immune and endocrine systems [8-11]. Tissue specific contamination and toxicokinetics are related to the physiology of the animals [12]. Differences in lipid distribution and lipid class profile, as well as lipid dynamics like fat accumulation and fasting, may affect tissue concentrations of POPs [4,13]. Weight loss in winter due to sub-maintenance feed intake is normal for e.g. free-living reindeer. A new equilibrium and redistribution of POPs in the body is established due to fasting and lipid loss and depending of the species, different tissues of animals can be targets for possible toxic effects [1]. In addition, milk production and lactation rely on fat depots, are significant route of elimination, and may therefore alter the distribution and decrease body burden of POPs in females [14].

Species-specific exposure, metabolism and accumulation of POPs result in different concentrations and contaminant profiles in the studied species. Ecophysiologocal factors, like feeding, may have an impact on the congener pattern seen in the animal body. E.g. PCDD/F profile in roe deer liver resembles that in conifer shoots (indicator for deposition via air), while profile in sheep liver is more similar with that of soil, indicating different eating behaviors [15]. Accumulation patterns may also vary among individuals of the same species, for example, Finnish reindeer fed only on natural pastures had higher PCDD/F- and DL-PCB concentrations than reindeer who had got supplementary feed [16]. Finnish reindeer and moose are interesting study objects because they share common living and contaminant deposition areas, and both are used as foodstuffs. Species-specific differences and elimination of dioxins in these economically, culturally and environmentally important Cervid species are highly interesting.

Age is considered to be one of the factors affecting POP levels. Generally, older individuals have higher POP levels than younger ones [17,18]. However, studies on reindeer in Finland have revealed higher concentrations in reindeer calf muscle than in adult reindeer muscle [19]. Accumulation of POPs in the early life stages of reindeer is supported by the observation of PCDD/Fs and DL-PCBs in Finnish stillborn reindeer calves [20]. Contaminant transfer from hind to fetus via placenta is occurring at critical and sensitive time period (body fat mobilization in mother and sensitive periods of organogenesis in fetus) resulting increased stress. Thyroid and steroid hormone systems are among the sensitive endocrine variables involved in the regulation of metabolic processes and development, and are potential targets of POPs both in hind and in fetus [21].

In addition to TEQs that are needed for risk assessment, the studies on individual congener profiles of PCDD/Fs and DL-PCBs in animal tissues give more detailed information on sources of exposure, species differences, individual variation, and differences among life stages and tissues. TEQ concentration, which is a measure of the total amount of PCDD/Fs and DL-PCBs adjusted for toxic potency, is a simplified method of assessing the risk of dioxin/PCB mixtures [22]. TEQ refers to the sum of the amounts of PCDD/Fs and DL-PCBs multiplied by their relative toxic potency as related to TCDD (the most toxic congener) according to the WHO [23].

The purpose of this study was to determine the concentrations and accumulation of 17 toxic PCDD/F congeners and 12 DL-PCB congeners in semi-domesticated reindeer and wild moose in order to identify the congener profiles of PCDD/Fs and DL-PCBs and reveal possible species- and tissue-specificity in accumulation. Standardized sampling methods were used to perform a survey of contaminant levels and profiles.

Methods: The sampling area of reindeer and moose located in the sub-arctic northern Finland (Figure 1) and covered the reindeer herding region. The region was divided into three different sampling zones; the northern, the middle and the southern zone. The method of sampling was standardized hence allowing a comparison of the results between...
the different zones. The samples were built up in a ratio of carcass meat consumption. Concentrations of 17 toxic PCDD/F and 12 DL-PCB (dioxin-like PCBs; 4 non-ortho and 8 mono-ortho congeners) were measured from each sample (Table 1).

**Reindeer and moose muscle samples:** The pooled muscle samples (weight 500 g) of reindeer (n=40) and moose (n=12) consisted of 200 g rump, 200 g rib and fore back, and 100 g shoulder muscle. The sampling was conducted using a clean knife and nitrile gloves to prevent contamination. The samples were stored in polyethylene bags in -20°C until analysis.

**Stillborn reindeer calves:** The muscle samples (on average 270 g) of stillborn reindeer calves (n=11) were collected from spontaneously aborted calves. The samples consisted of rump, back and shoulder muscles. Brown adipose tissue (BAT) samples (n=3) were taken from stillborn calves of the middle and southern sampling zones. BAT samples were collected from the specific locations; around the shoulders, sternum, trachea and spinal cord, and from the abdominal and thoracic cavities. The samples weighted on average 20 g.

**Reindeer liver samples:** The reindeer livers (n=14) were taken as solid tissues from reindeer calves and adult reindeer by using a clean knife and nitrile gloves to prevent contamination. The samples were stored in polyethylene bags in -20°C until analysis.

**Reindeer milk samples:** The reindeer milk samples were gathered in the Kaamanen experimental reindeer station in Inari, localizing in the northern sampling zone. Milk samples were collected twice from the reindeer hinds (n=7), in the early summer and later in the autumn. The sample collection (30 ml in each) was performed by hand milking to pre-cleaned glass bottles. The milk collection was facilitated by using Oxytocin (10 IU, l.m.) to each hind. Milk samples were preserved frozen (-20°C) in dark until analysis.

**Chemical analysis:** The analyses were performed at the accredited reference laboratory of chemistry at the National Institute for Health and Welfare in Finland. The requirements of standard EN ISO/IEC 17025 were completed. After homogenization the samples were freeze dried and fat was extracted with ethanol-toluene using Accelerated Solvent Extractor (ASE 300) equipment. The solvent was exchanged to hexane and the fat content was determined gravimetrically. The samples were defatted on an acidic silica column and purified and fractionated on alumina and carbon columns. PCDD/Fs and PCBs were analyzed with HRGC/HRMS using a selected ion monitoring mode (SIM) and resolution of 10 000. Further details of the analytical method can be found elsewhere [20].

**Reporting of the results:** WHO-TEQ concentrations are reported as fat based upper bound concentrations (concentrations<LOQ=LOQ) to enable comparisons to the existing EU maximum level in the meat [24]. The most abundant congener-specific PCDD/F and DL-PCB concentrations are reported as lipid based lower bound concentrations (concentration < LOQ = 0). Blank samples covering the whole analytical procedure did not indicate any cross contamination.

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**Table 1:** Analyzed PCDD/Fs and DL-PCBs with toxic equivalent factors

<table>
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<th>TCDD equivalent</th>
<th>TCDD equivalent</th>
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**Figure 1:** Sampling area.
Figure 2 (abstract S11) WHO-PCDD/F- and WHO-PCB-TEQs (pg/g fat) in a.) reindeer and moose muscle samples, and b.) in reindeer liver and brown adipose samples. *[16], **[20]. No=Northern zone, Mi=Middle zone, So=Southern zone. BAT=Brown adipose tissue.

Figure 3 (abstract S11) The most abundant PCDD/F congeners (pg/g fat) in a.) reindeer and moose muscle samples, and b.) in reindeer liver samples. No=Northern zone, Mi=Middle zone, So=Southern zone.
Results and discussion: PCDD/Fs in reindeer and moose tissue samples: WHO-PCDD/F-TEQs in reindeer muscle (Figure 2 a) were quite equal (on average 1.2 pg/g fat) in the different sampling zones, being only moderately higher in the southern zone, and generally higher in the calves than in adults. WHO-PCDD/F-TEQs in reindeer liver (Figure 2 b) followed the same pattern than in muscle: higher levels in the calves and in the southern zone. The most prominent PCDD/F congeners in reindeer muscle samples were 23478-PCDF, 123678-HxCDD and OCDD (Figure 3 a), of which 23478-PCDF and OCDD were also characteristic for reindeer liver samples. In addition, 123478-HxCDF, 123678-HxCDF, 234678-HxCDF and 1234678-HpCDF were well representative in liver (Figure 3 b). For the comparison, some of these congeners found in reindeer liver, namely 23478-PCDF, 1234678-HpCDF and OCDD, have been the most common congeners in roe deer liver samples in Germany [15]. Despite of the different zoogeographical origin of the species, roe deer’s feeding behavior resembles that of reindeer: variety of grasses, lichens, mushrooms, twigs and branches.

In this study, OCDD, with relatively low toxicity (TEF 0.0001) had a special property to exist at high concentrations (up to 140 pg/g fat) in reindeer muscle and liver in the northern area calf samples. This may indicate special and different exposure to OCDD from some wide scale emission source in the northern Finland, considering that adult reindeer in the same area also had proportionate high OCDD concentrations, especially in their liver. Also 2005 sampled reindeer calves in North-East Finland have shown high (160-fold) OCDD concentrations in muscle compared to many other existing PCDF/F congeners. The more toxic PCDD/F congener, 23478-PCDF (TEF 0.5) concentrations were high in the calves and adult reindeer of the southern area, especially in their livers. This congener showed also high contribution in stillborn calves’ muscle tissue in the same area, and also in middle area’s stillborn calf muscle. Statistically significant differences (P<0.05) were seen in the concentrations of 2378-TCDF, 123478-HxCDD and 1234678-HpCDF, which showed to exist in higher levels in the calf, especially in stillborn calf, muscle samples in every sampling zone. On the contrary, 12378-PCDF concentrations were significantly (P<0.05) higher in adult reindeer muscle samples. WHO-PCDF/F-TEQs in stillborn calves’ muscle were slightly higher in the middle and southern zone (on average 2 pg/g fat) than in the northern zone (0.8 pg/g fat).

Considering stillborn calves’ brown adipose tissue, it was seen that mean 23478-PCDF concentration (2.4 pg/g fat) was overwhelmingly highest of any congeners (data not shown), and thus affected strongly (TEF=0.5) to the WHO-PCDD/F-TEQs, which were 1.7 and 4.1 pg/g fat in the studied middle and southern areas (Figure 2 b). However, also many other PCDD/F congeners existed in brown adipose and three of them (2378-TCDD, 123478-HxCDD and 1234678-HpCDF) were lacking in muscle samples but represented in the brown adipose. On the other hand, 2378-TCDF, 1234678-HpCDF and OCDD seemed to exist in the muscle samples, but not in brown adipose, of stillborn reindeer calves.

Highly chlorinated congeners like 1234678-HpCDF and OCDD are generally considered to accumulate well in lipid-rich tissues, like brown adipose (fat content on average 30%), so the lack of these congeners is interesting. It may be the strong binding of these high chlorinated congeners to reindeer hind’s fat storages that restricts the transplacental diffusion to brown adipose of fetus. However, the muscle of fetus contained 1234678-HpCDF and OCDD in the middle and southern zones that may be indication of individual variations. There are indications of tissue-specific retention of PCDD/Fs in animals [25] that may be caused by structural specific binding sites and relative degree of absorption; those may partly explain different profiles in different areas and exposure conditions. 1234678-HpCDF and OCDD have observed to be the major PCDD congeners in the deposition samples [26], in northern Finland (26). WHO-PCDF/F-TEQs in moose muscle (Figure 2 a) (on average 1.3 pg/g fat) were equal with reindeer. The most dominating PCDD/F congeners in moose muscle tissue samples were OCDD, 23478-PCDF, 1234678-HpCDF and 1234678-HpCDD (Figure 3 a). OCDF concentration (28 pg/g fat) was noticeable high in male calf sample from the northern zone. That was a parallel result to the reindeer calf muscle and liver samples from the same sampling zone, and indicated high OCDD exposure in the northern part of Finnish Lapland. Also 1234678-HpCDF showed an elevated concentration in northern zone’s moose male calf. A similar phenomenon was observed in reindeer calves from the northern zone.

Lower OCDD and 1234678-HpCDF concentrations in northern zone’s adult reindeer and adult moose may indicate different contaminant levels in emissions and exposure from food, in addition to individual physiological differences, but unlikely straight differences in metabolic activity and elimination potential. In middle and southern zones the exposure to especially OCDD may be lower and that is reflecting as the lower contamination both calves and adults. Concentration of 2378-TCDF was significantly (P<0.05) higher in the adult moose than in moose calves. That was an opposite result than with reindeer. Very similar concentrations of 23478-PCDF, 123478-HxCDF, 123678-HxCDF and 234678-HxCDF (TEF 0.1 in hexa-chlorinated congeners) in the livers of the southern zone’s reindeer calves and adult reindeer could indicate equal amount of binding sites for dioxins in the liver. On the other hand, considering the concentrations of the same congeners in the northern and middle zones, it is seen that calves had general higher levels in their livers than adult reindeer. However, the total concentrations were lower in the northern and middle zones than in the southern zone, even though the differences were not statistically significant.

Table 2(abstract S11) PCDD/Fs (pg/g fat), non-ortho-DL-PCBs (pg/g fat), and mono-ortho-DL-PCBs (ng/g fat) in reindeer milk samples

<table>
<thead>
<tr>
<th>Congener</th>
<th>Summer milk</th>
<th>Autumn milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>2378-TCDF</td>
<td>0.011</td>
<td>0.076</td>
</tr>
<tr>
<td>2378-TCDD</td>
<td>0.010</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>12378-PCDF</td>
<td>0.033</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>23478-PCDF</td>
<td>0.260</td>
<td>0.143</td>
</tr>
<tr>
<td>12378-PeCDF</td>
<td>0.143</td>
<td>0.016</td>
</tr>
<tr>
<td>123478-HxCDF</td>
<td>0.160</td>
<td>0.044</td>
</tr>
<tr>
<td>123678-HxCDF</td>
<td>0.063</td>
<td>0.024</td>
</tr>
<tr>
<td>234678-HxCDF</td>
<td>0.046</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>123789-HxCDF</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>1234678-HxCDF</td>
<td>0.124</td>
<td>0.023</td>
</tr>
<tr>
<td>12389-HxCDF</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>1234678-HpCDF</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>1234789-HpCDF</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>1234678-HpCDD</td>
<td>0.098</td>
<td>0.126</td>
</tr>
<tr>
<td>ODCDF</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>OCDD</td>
<td>&lt;LOQ</td>
<td>0.589</td>
</tr>
<tr>
<td>PCB-77</td>
<td>&lt;LOQ</td>
<td>0.970</td>
</tr>
<tr>
<td>PCB-81</td>
<td>0.062</td>
<td>0.364</td>
</tr>
<tr>
<td>PCB-126</td>
<td>5.070</td>
<td>2.653</td>
</tr>
<tr>
<td>PCB-169</td>
<td>0.798</td>
<td>0.511</td>
</tr>
<tr>
<td>PCB-105</td>
<td>0.176</td>
<td>0.122</td>
</tr>
<tr>
<td>PCB-114</td>
<td>0.012</td>
<td>0.008</td>
</tr>
<tr>
<td>PCB-118</td>
<td>0.404</td>
<td>0.390</td>
</tr>
<tr>
<td>PCB-123</td>
<td>&lt;LOQ</td>
<td>0.005</td>
</tr>
<tr>
<td>PCB-156</td>
<td>0.091</td>
<td>0.060</td>
</tr>
<tr>
<td>PCB-157</td>
<td>0.015</td>
<td>0.008</td>
</tr>
<tr>
<td>PCB-167</td>
<td>0.025</td>
<td>0.022</td>
</tr>
<tr>
<td>PCB-189</td>
<td>0.009</td>
<td>0.009</td>
</tr>
</tbody>
</table>
(0.5 pg/g fat) to autumn sampling (0.4 pg/g fat) [20]. The most dominating congeners were 23478-PeCDF, 12378-PeCDD, 123478-HxCDF, 123678-HxCDD, 1234678-HpCDD and OCDD. There was a decreasing trend in reindeer milk samples from summer to autumn in most of the congener concentrations. Statistically significant ($P<0.05$) decrease were in the concentrations of 23478-PeCDF, 12378-PeCDD, 123478-HxCDF, 123678-HxCDD and 123678-HpCDD). However, 2378-TCDF, 1234678-HpCDD and OCDD showed significantly higher levels in the autumn than in the summer. In addition, OCDD, which showed the highest total contribution of PCDD/Fs, interestingly increased from zero in summer to on average 0.6 pg/g fat in autumn milk samples. Despite of increasing fat-content of milk during the lactation period, the levels of some particular congeners were increased indicating no dilution effect.

**DL-PCBs in reindeer and moose tissue samples:** WHO-PCB-TEQs were higher in reindeer calf muscle (Figure 2 a) (on average 2.0 pg/g fat) than in adult reindeer (on average 1.2 pg/g fat). The highest level was seen in the middle sampling zone. The most dominating non-ortho-DL-PCB congener in reindeer muscle samples (Figure 4 a) was PCB-126, which highest concentration (39 pg/g fat) was detected in the middle zone's stillborn calf. That individual contained also quite high concentration of PCB-77 (18 pg/g fat), which was the other very frequent congener in the studied population. PCB-77 was the only non-ortho congener being significantly ($P<0.05$) higher in stillborn calves’ muscle than in the other calves and adult reindeer. PCB-77 had also significantly ($P<0.05$) higher levels in stillborn calves’ muscle than in brown adipose tissue.

From non-ortho congeners, PCB-126 was the most dominating one in brown adipose (data not shown), followed by PCB-169. In addition, even if PCB-169 existed in many of the samples, it showed the highest level in the stillborn calf of the middle area (14.4 pg/g fat) (Figure 4 a). WHO-PCB-TEQ in stillborn calves’ brown adipose (on average 5.2 pg/g fat) was higher than in muscle (on average 2.6 pg/g fat). Overall, there was a strong contribution of dioxin-like non-ortho-PCBs (PCB-77, -81, -126 and -169) to total TEQ in the reindeer muscle samples. Of the mono-ortho-PCBs, PCB-118 was the most generally existing congener. In 38% of reindeer and moose tissue samples its concentration was above 1 ng/g fat (Table 3). The most congener containing mono-ortho-PCB profile was detected in middle area’s stillborn reindeer calf that indicates effective transfer and accumulation of these contaminants to fetus.

PCB-126 was clearly the most dominating DL-PCB congener in reindeer liver samples (Figure 4 b), followed by other non-ortho-DL-PCB congeners PCB-77, -81 and -169, which concentrations were, however, much lower. The highest concentration of PCB-126, 400 pg/g fat, was exceeded in the middle zone’s female calf. TEF-value of PCB-126, 0.1, is the highest of DL-PCBs, thus influencing strongly to WHO-PCB-TEQ, which was higher in reindeer calf liver (on average 29 pg/g fat) than in adult reindeer liver (on average 12 pg/g fat) (Figure 2 b). The overall non-ortho-DL-PCB profile in reindeer liver fitted well to reindeer muscle samples. DL-PCB concentrations were generally higher in calf liver samples than in adult reindeer liver. That may indicate the calf liver functions being in state of effective accumulation of toxicants and weak detoxification resulting high concentrations of non-metabolized compounds in calf livers. However, concentrations of PCB-77 were significantly ($P<0.05$) higher in adult reindeer livers than in calves’ livers.

WHO-PCB-TEQ in moose calf muscle (Figure 2 a) was slightly lower (0.7 pg/g fat) than in adult moose (0.9 pg/g fat), that is opposite result than with reindeer. The most prominent non-ortho congener in moose muscle was PCB-77, followed by PCB-126 and PCB-81. Of mono-ortho-PCB congeners PCB-118 was the most detected one. There were no statistically significant differences between the DL-PCB concentrations in adult moose and moose calves. However, PCB-77 concentrations were significantly lower in female moose than in male moose in every zone, indicating excretion of compounds via lactation.

**DL-PCBs in reindeer milk samples:** The most prominent non-ortho-DL-PCB congener in reindeer milk (Table 2) was PCB-126; this concerns both summer and autumn samples, when the mean concentrations were 5 and 2.7 pg/g fat, respectively. A clear decrease in the concentration of

![Figure 4](abstract S11) Non-ortho-DL-PCBs (pg/g fat) in a) reindeer and moose muscle samples, and b) in reindeer liver samples.
PCB-126 is seen from summer to autumn. Similar significant, (P<0.05) decreasing trend was seen also with non-ortho-PCB-169 (0.79 pg/g fat) in summer and 0.51 pg/g fat in autumn). However, PCB-81 increased significantly (P<0.05) from 0.06 pg/g fat in summer to 0.36 pg/g fat in autumn, and PCB-77 from zero in summer to 0.97 pg/g fat in autumn. This was due to one exceptional high PCB-77 concentration (6.79 pg/g fat) in autumn milk samples. PCB-118 remained steady (0.4 ng/g fat). Concentrations of PCB-114, -156 and -157 were decreased and PCB-123 increased significantly (P<0.05) from summer to autumn, concentrations being nevertheless very low.

The congener profile of DL-PCBs in reindeer milk was again fitted well to reindeer muscle and liver samples. Especially the most toxic DL-PCB-126 was well represented. WHO-PCB-TEQ in summer milk samples, on average 0.6 pg/g fat, decreased to 0.4 pg/g fat in autumn [20] that indicates transfer of DL-PCBs out of the body of female reindeer via lactational route. However, even emphasizing the importance of lactational transfer of persistent organic compounds it is worth of noticing that the highest concentrations of PCB-126 and -169 were found from stillborn calf that had got its body burden only via the placenta. The mean fat content in autumn milk samples (26%) was significantly (P<0.05) higher than in summer milk samples (10%) that may has an influence to the lipid based concentrations detected. The fat content of reindeer milk normally varies from 11 to 30% during the lactation process [27].

Conclusions: WHO-PCDD/F-TEQs were generally higher in reindeer calf muscle than in adult reindeer. PCDD/Fs showed partly similar profiles in reindeer and mouse muscle, reindeer liver and milk samples-indicating equal mode of bioaccumulation. Among the most abundant congeners were 23478- PeCDF, 123478-HxCDF, 123678-HxCDF, 234678-HxCDF, 1234678-HpCDD and OCDD. However, stillborn calves’ brown adipose was somewhat exception showing both accumulation and absence of congeners compared to muscle. In addition, more infrequent PCDD/F congeners existed in moose muscles and in adult reindeer. In addition, more infrequent PCDD/F congeners compared to muscle. In addition, more infrequent PCDD/F congeners were noted in adult reindeer.

List of abbreviations used: HRGC/HRMS: High resolution gas chromatography/high resolution mass spectrometer; WHO-TEQ: Toxic equivalent defined by WHO; WHO-PCDD/F-TEQ: Toxic equivalent for 17 PCDD/Fs; WHO-PCB-TEQ: Toxic equivalent for 12 DL-PCBs; TEF-value: Toxic Equivalence Factor; LOQ: Limit of quantification.

Competing interests: The authors declare that they have no competing interests.

Authors’ contributions: Study design: SL. Data collection: SL, MN. Analysis of samples: PR. Data analysis: AS, SL. Statistical analysis: AS. Manuscript writing: AS. Critical review and approval of the final manuscript: AS, AH, PR, HK, MN, SL.

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Park Bjørngaard
Institute of Biology, University of Southern Denmark, Campusvej 55, DK-5230
Odense M, Denmark

Sex hormones produced in humans and livestock are excreted in the urine.
Naturally produced and synthetic contraceptive pill estrogens may pass low-
quality water processing plants and feminize male fish in streams and
coastal areas downstream such discharges [1,2]. High-quality processing of
the wastewater with tertiary treatment, nutrient removal and sufficient
water and sludge retention times basically removes the problem [3,4].
The feminization of male fish downstream waste water discharges has
already been demonstrated by the presence of elevated levels of female
yolk protein (vitellogenin) in the blood of male or juvenile fish or eggs
(or oocytes) in the testes (intersex) [5]. Both of these phenomena are
used as biomarkers for estrogenic effects and as always in the use of
biomarkers, it is important to define the natural background level in an
uncontaminated environment.
The first investigations on intersex in roach showed that male fish in
areas not directly affected by wastewater discharges had intersex
percentages between 5 and 11% [1,6], suggesting that there might be a
low, natural occurrence of this phenomenon. Recent investigations [7]
in pristine areas do, however, show no intersex among the males, indicating
that the natural background level of intersex roach may actually be zero.
Plasma levels in male or juvenile fish have been used as an efficient
biomarker to detect estrogenic contamination in both freshwater and
marine areas and a number of studies [e.g. [8,9]] have presented
vitellogenin levels that are markedly and unambiguously elevated - maybe
several orders of magnitude - compared to background levels. Less effort
has been devoted to defining when less severe increases in plasma
vitellogenin levels actually indicate a difference relative to unaffected
background levels [10]. The occurrence of intersex in roach and elevated
vitellogenin levels in areas not directly affected by discharges from sewage
treatment plants suggests that there may be other inputs of estrogenic
activity to the aquatic environment and, in fact, several possibilities – some
of them associated with intensive, modern agriculture - exist.
Estrogens have been found to leach to the water draining from fields
treated with pig manure [11], which generally has a high content of
especially estrone and estradiol activity has been found in streams in
connection with dairy cattle farms [12].
Leguminous plants - such as clover, peas, lupine and alfalfa used as
nitrogen binding crop in e.g. organic farming - produce phytoestrogens
which may leach to - and are detected in - the freshwater environment
[13] – most often in the ng/l range. Some phytoestrogens induce
vitellogenin synthesis in the low µg/l range [14].
Houses in the open land are not all connected to central sewage
treatment plants and simple waste water processing measures such as
septic tanks may be employed. Septic tanks do a very poor job removing
estrogens from wastewater and estrogenic activities 20 times higher than
needed to feminize brown trout have been detected in discharges from
such systems [15]. Recently, exposure to 17ß-estradiol and octylphenol has been shown to
cause malformations in embryos of viviparous eelpout at fairly high – but
still environmentally realistic concentrations [16].

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513 Developmental toxicity of pharmaceuticals in lower vertebrates
Cecilia Berg, Björn Brunström, Ingvar Brandt
Dept of Environmental Toxicology, Uppsala University, Sweden

The release of human pharmaceuticals in the aquatic environment has become an issue of societal concern. More than 160 Active Pharmaceutical Ingredients (APIs) have been detected in surface waters, although generally at low concentrations (ng-μg/l). Most available ecotoxicological information on these pharmaceuticals originates from acute testing using algae, Daphnia and fish; results from long-term exposures are still very scarce, particularly in vertebrates. There is, however, emerging evidence to suggest that several human and veterinary pharmaceuticals may pose a serious threat to aquatic wildlife. Still, there are only two examples where exposure to pharmaceuticals has been indisputably linked to adverse effects in wild vertebrates, i.e. the feminizing effects of the synthetic estrogen ethinylestradiol (EE2) in fish and the decline in vulture populations caused by the non-steroidal anti-inflammatory drug diclofenac in India and Pakistan.

We are currently studying effects of steroid hormone APIs on sex organ development following exposure during early life-stages in birds (egg injection), frogs and fish (exposure via ambient water). The results show that the developing Müllerian duct (absent in fish) is a highly sensitive target for endocrine disruption and developmental toxicity in amphibians and birds.

In birds, both diethylstilbestrol (DES) and EE2 injected into the embryonated egg cause a disrupted differentiation and malformations in different parts of the oviduct, including the shell gland. Egg injection of EE2 also resulted in eggshell thinning in the next generation of eggs, and a dramatically altered pattern of expression of the enzyme carbonic anhydrase in the shell gland. Eggshell thinning is probably the most serious ecotoxic effect that has afflicted avian wildlife. Based on these and other data, we propose that eggshell thinning could represent a developmental effect in the Müllerian duct rather than a direct effect of pollutants in the oviduct of the adult egg-laying bird.

The Müllerian duct also proved to be a sensitive target of toxicity in frogs. Exposure of tadpoles to EE2 resulted in a skewed sex ratio with almost complete feminization at an ecologically relevant concentration. A partial or complete lack of oviducts was observed in these frogs, resulting in ovulation directly into the abdominal cavity. Also the gestogenic pharmaceutical levonorgestrel (LNG), used in contraceptives and other hormone therapies, has recently been demonstrated to give rise to oviductal agenesis following developmental exposure. Unlike the impact in EE2-exposed frogs, the adult ovaries of the LNG-exposed frogs also displayed an increased fraction of immature oocytes, arrested in early meiotic prophase. We conclude that both EE2 and LNG are potent developmental toxicants at environmentally relevant exposures in frogs, giving rise to dysfunctional female sex organs with subsequent sterility.

In conclusion, exposure to pharmaceutical steroids during early life stages can permanently impair female reproductive organ development and fertility in lower vertebrates.

514 Fish models for ecotoxicology
Leif Norgren
Department of Biomedicine and Veterinary Public Health, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden

Background: Development of fish models for assessment of chemicals, which may interfere with different parts of the life cycle, act over consecutive generations and with a potential to have impact on populations, are essential for risk assessment and environmental protection.

1. Laboratory models: Fish laboratory models covering different parts of the life cycle and different routes of exposure are continuously developed and modified in order to meet new challenges. The most important exposure routes are by injection, through contaminated water or feed [1]. For laboratory studies zebrafish (Danio rerio), medaka (Oryzias latipes), fathead minnow (Pimephales promelas) and three-spined stickleback (Gasterosteus aculeatus) are key species proposed by OECD. Examples of laboratory models are:

- Fish Embryo Assays (FEAs) are used to study early developmental disorders. Exposure is performed from fertilisation to hatching. A number of developmental parameters are included, i.e. stage, somite formation, heart rate, eye development, pigmentation, tail lifting, hatching, behaviour etc. FEAs have been used to evaluate for instance musks [2], effluents waters from the production, pharmaceuticals [3] and waste products [5].

- The Fish Sexual Developmental Test (FSDT) has been developed in Nordic countries to evaluate the impact on Endocrine Disrupting Chemicals (EDCs) on sex differentiation and sex ratios. The FSDT has recently been accepted as an OECD guideline. The FSDA has been shown that laboratory fish species show changed VTG concentrations and skewed sex ratios after exposure to estrogens or androgens [6-8].

- The Fish Screening Assay (FSA) is similarly to the FSDA primarily developed to evaluate EDCs. This assay is initiated by a preexposure period to confirm normal reproductive capacity. Exposure is performed trough water or feed during at least 21 days. The exposure period is
followed by evaluation for instance of reproductive fitness, fecundity and fate of the offspring [9,10].

-Fish Full Life Cycle Assays (FFCAs) covers exposure during at least two consecutive generations. FFCAs are the ultimate models to predict risks for populations; however, these long-term exposure models are very expensive to perform and further evaluation is necessary in order to confirm robustness and to define suitable endpoints.

2. In situ models: Besides laboratory assays more complex tests can be performed by using in situ exposure models by deployed cages. These models are used to determine for instance offshore oil production, impact of remediation activities [11] and sewage/industrial effluents.

3. Mesocosm models: Mesocosm models have the advantage compared to laboratory and in situ models that these maintain a natural ecosystem community close to natural conditions and thereby mimicking a real ecosystem. Mesocosm models can be either land- or water based. Interactions between algae, invertebrates and fish can be assessed.

Conclusions: Fish models to assess the impact of chemicals are continuously developed in order to introduce more sensitive and relevant endpoints. Today, this development includes a panorama of methodologies such as gene expression, proteomics, physiological biomarkers, pathology, reproduction and behaviour.

References

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Two decades of biomonitoring polar bear health in Greenland: a review

Christian Sonne1*, Robert J Letcher2, Thea Ø Bechshøft3, Frank F Rigét4, Derek CG Muir5, Pall S Leifsson6, Erik W Born7, Lars Hylstra˚7, Niladri Basu7, Maja Kirkegaard8, Rune Dietz9

1Department of Bioscience, Faculty of Science and Technology, Aarhus University, Frederiksbergvej 399, PO Box 358, DK-4000 Roskilde, Denmark; 2Aquatic Ecosystem and Health Division, Environment Canada, National Wildlife Research Centre, Carleton University, Ottawa, Ontario, K1A 0H3, Canada; 3Aquatic Ecosystem Protection Research Division, Environment Canada, Burlington, Ontario, L7R 4A6, Canada; 4Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Bülowsvej 17, DK-1870 Frederiksberg, Denmark; 5Greenland Institute of Natural Resources, PO Box 570, DK-3900 Nuuk, Greenland, Denmark; 6University Hospital of Hvidovre, Kettegaard Alle 30, DK-2650 Hvidovre, Denmark; 7Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI, USA; 8Danish Agency for Science, Technology and Innovation Bredgade 40 DK-1260 Copenhagen K, Denmark; E-mail: csh@dmu.dk


Background: The use of East Greenland polar bears (Ursus maritimus) as a biomonitoring key species for measuring exposure to OHCs (organohalogens compounds) and Hg (mercury) was initiated in Scoresby Sound in 1983 and is now the most comprehensive time trend studies of pollution on this species [1-7]. In addition, museum samples of skin and skulls collected since year 1892 have been included for Hg analysis and various patho-morphological and toxico-pathological density analyses [4,6-11].

The studies of adverse health effects of pollution were started in 1999 by the implementation of a biomonitoring health programme via AMAP (Arctic Monitoring and Assessment Programme) [12-14]. The reason for choosing polar bears, and for upgrading the research intensity, was because the East Greenland polar bear subpopulation is one of the most contaminated and that local Inuit people rely on this species as a food resource in addition to ringed seal (Phoca hispida) that plays a much greater role. Because polar bears reflect temporal trends and biological effects of contaminants they may also serve as a proxy for human health exposure and possible effects despite the fact that the physiology, metabolism, food and way of life of these two species differ fundamentally [e.g. [5,7,13-15]].

We present an overview of contaminant concentrations and potential adverse health effects from anthropogenic contaminants in polar bears during the period 1999-2010. The health effects include decrease in bone density, morphological changes in sexual organs, liver, kidney and thyroid glands, as well as potential neurological alterations and impacts on the immune and endocrine systems. Finally, some speculations about the synergistic effects of environmental stressors (e.g. decrease in sea ice) on polar bear health are presented.

Levels of contaminants: Chlorinated legacy contaminants (PCBs and OC pesticides), brominated flame retardants (PBDEs), perfluorokalkyl contaminants (PFCs) and Hg have been analyzed in brain, adipose tissue, liver, kidney, blood and hair samples from East Greenland polar bears. The range of contaminant concentrations used in relation to health endpoints during the period 1999-2002 are seen in Table 1. Concentrations increase in the order: PBDEs>PFCs>PCBs>Hg and are in the concentration of having adverse health effects according to the international scientific literature [13,14,16,17].

According to Dietz et al. [3,5,7] the concentrations of legacy chlorinated contaminants (PCBs and OC pesticides) have decreased and stabilized since 1999, while newer contaminants, like PFCs and contemporary threats like Hg have increased. So, despite international regulations on all these groups of contaminants they persist and biomagnify in the environment, which results in a cocktail of toxic chemicals in the tissues of East Greenland polar bears.

Skeletal system: Analyses on the skeletal system have exclusively focused on skulls. The reasons for this are because Natural History Museums have archived these since 1892 and because skulls are relatively easily obtained in connection with the subsistence hunt. We examined skull bone mineral density (BMD) in 139 bears in the period 1892-2009 and after controlling for age and sex we found that BMD decreased significantly over time in subadults (Figure 1) and adult males but not in adult females (data not shown) [18]. That a similar decrease was not found in adult females was likely due to the fact that they increase bone density prior to denning in order to avoid demineralisation. Otherwise, the low mechno-transduction and limited food intake and high calcium flux via foetal development and lactation transfer during denning theoretically should lead to clinical osteoporosis [10].

When correlating BMD with individual body burdens of OHCs in adipose tissue for individuals sampled during 1999-2002, significant inverse relationships were found in subadults (Figure 2) and adult males but not females [18]. Similar relationships were found for bacular BMD [20]. Furthermore, a calculation of T-score in males showed their risk of developing osteoporosis [14] as neuro-signalling may be influenced due
Table 1 (abstract S15) Concentrations of various contaminants [Mean (Min-Max, n)] divided on tissues in East Greenland polar bears sampled 1999-2002. All data are in ng/g lw except for nPCBs, PCDDs and PCDFs (pg/g lw), PFCs (ng/g ww) and mercury (µg/g ww). Reworked from Sonne (2010).

<table>
<thead>
<tr>
<th>Subcutaneous adipose tissue</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid-%</td>
<td>88</td>
<td>11</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>∑PCB</td>
<td>6,543 (897-20,407, 92)</td>
<td>28,409 (12,836-67,664, 20)</td>
<td>-</td>
<td>148-2,186 (20)</td>
</tr>
<tr>
<td>HCB</td>
<td>102 (2.4-785, 92)</td>
<td>109 (20)</td>
<td>-</td>
<td>15 (20)</td>
</tr>
<tr>
<td>∑HCH</td>
<td>194 (13-818, 92)</td>
<td>67 (20)</td>
<td>-</td>
<td>15 (20)</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>204 (26-866, 92)</td>
<td>4,900 (20)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>∑Chlordane</td>
<td>1,414 (243-7,465, 92)</td>
<td>37,400 (20)</td>
<td>-</td>
<td>62 (20)</td>
</tr>
<tr>
<td>∑DDT</td>
<td>436 (73-1,580, 92)</td>
<td>&lt;0.1-476 (20)</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>∑PBDE</td>
<td>70 (22-192, 92)</td>
<td>127-936 (20)</td>
<td>-</td>
<td>&lt;0.5-36 (20)</td>
</tr>
<tr>
<td>∑PFC</td>
<td>-</td>
<td>1,056-8,010 (29)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>∑PCB</td>
<td>241 (125-442, 5)</td>
<td>124 (114-148, 5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>∑PCDD</td>
<td>10 (7-12, 5)</td>
<td>20 (8-38, 5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>∑PCDF</td>
<td>4 (3-5, 5)</td>
<td>14 (10-18, 5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>∑CH/MeSO₂-PCB</td>
<td>90-3134 (20)</td>
<td>1,882-18,018 (20)</td>
<td>-</td>
<td>66-352 (20)</td>
</tr>
<tr>
<td>∑CH/MeSO₂-PBDE</td>
<td>&lt;0.3-39 (20)</td>
<td>&lt;0.5 (20)</td>
<td>-</td>
<td>&lt;0.5 (20)</td>
</tr>
<tr>
<td>∑MeSO₂-P,p’-DDE</td>
<td>90-3134 (20)</td>
<td>1,882-18,018 (20)</td>
<td>-</td>
<td>66-352 (20)</td>
</tr>
<tr>
<td>Mercury</td>
<td>-</td>
<td>11 (1-36, 59)</td>
<td>14 (1-50, 57)</td>
<td>0.4 (0.1-0.9, 82)</td>
</tr>
</tbody>
</table>

Figure 1 (abstract S15) Bone mineral density (g×cm⁻²) as a function of sampling year in subadult East Greenland polar bears sampled 1892-2002. R² = 0.31** (n=50).
chlorinated and brominated groups of contaminants as well as the liver and renal toxic Hg concentrations. No histological lesions were found in any of the 50 adrenals examined while pathology was found in 8 out of 20 examined thyroid glands [28]. None of the thyroid gland lesions including c-cell hyperplasia, interstitial fibrosis, and nodular hyperplasia were associated with age or gender, so environmental factors such as energetic stress and autoimmunity/genetic could be co-factors as well as OHCs. Such lesions may interfere with the hypothalamic–pituitary–thyroid (HPT) axis leading to endocrine disruptions having an impact on fecundity and foetal and neonatal development in East Greenland polar bears.

Neuro-endocrine system: During 1999-2002 we sampled the medulla oblongata from 82 specimens in order to analyze the concentrations of Hg, a known neuro-toxicant. The analyses showed that the concentrations of Hg were relatively low compared to liver and kidney burdens, as well as other species, probably due to high demethylation capacity of the liver, formation of Hg-selenium complexes, and fur as an efficient excretion route [29]. Despite the low Hg concentrations some inverse correlations of statistical significance were found. First, like in several other wildlife species [30,31], Hg-associated decreases in the levels of NMDA receptor were found. The NMDA-receptor facilitates the neurotransmission of glutamate and is important for learning and Hg (Figure 4). Second, DNA methylation seemed to decrease with increasing Hg concentrations indicating potential epigenetic alterations in gene expression [32]. Villanger et al. [33] analyzed the circulating full blood concentrations of TT3 and TT4 in 62 East Greenland polar bears. The reason for analyzing thyroid hormones is the fact that this endocrine system is known to be extremely susceptible to EDC exposure including biotransformed metabolites such as OH-PCBs [13,14,33]. The analyses showed that various biological parameters such as age, size, and condition affected the circulating concentrations while different organochlorine contaminants and bromated flame retardants had negative as well as positive effects on the concentrations of both TT3 and TT4. The conclusions were that these correlations indicate biological effects on the HPT axis from EDC exposure, which is also supported by various in vivo studies of laboratory mammals as well as in vitro studies of polar bears [13,14,33].

Immune system: Very little has been published on the East Greenland polar bear immune system [13,14,34]. However, investigations on Svalbard bears have indicated immune toxic effects at OHC exposure concentrations similar to the East Greenland polar bear’s [13,14,35,36] and multiple studies in the laboratory and field show similar biological effects [13,14,37-39].

Future challenges: As shown above, contaminant exposure is suggested to have potential health effects on various organ-systems in East Greenland polar bears. However, contaminants are not the only environmental stressor in East Greenland (Figure 5). Also global warming leading to decreased food access and negative energy balance may influence bear health via (sub)clinical impacts on immune functioning and reduced fecundity having an impact on populating size [13,14,40-42]. In addition to this global warming may also increase the infectious stress due to invasive micro pathogen and parasitic diseases [13,14]. The main challenge in the future is therefore to integrate the cumulative impact

Figure 2(abstract S15) Bone mineral density (g×cm⁻²) as a function of adipose tissue PCBs (ng/g lw) in subadult East Greenland polar bears sampled 1999-2002. R²- and p-values from a full multiple regression model controlling for age. *: p < 0.05.

Figure 3(abstract S15) Testes length (mm) as a function of adipose tissue chlordane concentrations (ng/g lw) in 39 East Greenland polar bears sampled 1999-2002. R²- and p-values from a full multiple regression model controlling for age. **: p < 0.01.
from these multiple stressors across temporal and spatial gradients by integrating empirical data and laboratory studies. The East Greenland polar bear seems an excellent biomonitoring organism for such.

Conclusions: East Greenland polar bears are among the most contaminated species on our globe in spite of their remote Arctic habitat. This sub-population inhabits Arctic biotopes being constantly under pressure from global warming and associated environmental changes. Anthropogenic environmental contaminants seem to be a co-factor in various organ-system lesions in East Greenland polar bears. This includes reduced bone density and sexual and reproductive organ size, thereby having potential impacts on individual health and population maintenance. On top of this, also global warming seems to affect polar bears via negative energy balances, which may have consequences for fecundity and immune resistance. The main challenge in the future is to integrate the cumulative impact from these multiple stressors across temporal and spatial gradients by integrating empirical data and laboratory studies.

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References

Biological and toxicological effects of non-dioxin-like PCBs
Matti Vilukka1, Leo TM van der Ven1, Dieter Schrenk1, Hellmuth Lilienthal1, Patrik L Andersson1, Krister Hallidin1,2, Helen Håkansson1
1Department of Environmental Health, National Institute for Health and Welfare (THL), Kuopio, Finland; 2Laboratory for Health Protection Research, National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands; 3Food Chemistry and Toxicology, University of Kaiserslautern, Kaiserslautern, Germany; 4Center of Toxicology, Institute for Prevention and Occupational Medicine (IPA), German Social Accident Insurance, Ruhr University of Bochum, Bochum, Germany; 5Department of Chemistry, Umeå University, Umeå, Sweden; 6Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 7Department of Environmental Toxicology, Uppsala University, Uppsala, Sweden

Background: Polychlorinated biphenyls (PCBs) are a group of 209 congeners with highly similar basic structure, but differing in the number of chlorines and chlorination pattern. They are potent, persistent and accumulative, and abundantly present in food and the environment. A group of 12 congeners has a dioxin-like planar structure and toxicological properties similar to dioxins and are therefore called dioxin-like PCBs (DL-PCBs). Their risk assessment is included in that of other DL compounds. The rest of the PCBs, with non-planar structure due to chlorine substitution at ortho position, have a different toxicological profile with possibly several different mechanisms. They are referred to as non-dioxin-like PCBs (NDL-PCBs), and their toxic effects have been, so far, poorly characterized because of contamination of several used NDL-PCBs batches with very potent DL impurities (EBSA, 2005: http://www.efsa.europa.eu/de/scdocs/doc/284.pdf). The aim of the ATHON (Assessing the Toxicity and Hazard of Non-dioxin-like PCBs Present in Food) project was to provide missing critical health hazard information, to clarify biological mechanisms underlying the various types of toxicity of NDL-PCBs and to evaluate these data from the risk assessment point-of-view.

Materials and methods: High purity model compounds PCB180 (2,2’,3,4,4’,5,5’-heptachlorobiphenyl) or PCB52 (2,2’,5,5’-tetrachlorobiphenyl) were given at several dose-levels to young adult rats for 28 days, and to pregnant female rats from gestation day 7 until weaning. Toxic effects and induction of xenobiotic metabolising enzymes were studied at the end of treatment and in the offspring using haematology, clinical chemistry, biochemistry, molecular biology, histopathology, neurobehavioural testing and tissue PCB-level analyses.

Results: The cytochrome P450 (CYP) induction profile of both NDL-PCBs in liver was clearly different from that of DL compounds and characteristic of constitutive active androstane receptor (CAR) and pregnane X receptor (PXR) agonists. Neither of them was hepatotoxic. Both congeners caused reduced levels of circulating thyroid hormones T4 and T3 in adult animals and at lower exposure levels also in pregnant females and in the offspring. The likely explanation for hypothyroidism is increased hepatic clearance due to induced UGT activity and displacement of thyroid hormones from their transport protein transthyretin. Changes in retinoid metabolism were observed both after adult and perinatal exposure. Neurobehavioural effects included altered open field behaviour in adult females (PCB180), impaired auditory function in male and female offspring (PCB180 and PCB 52) and altered sexually dimorphic behaviour in female offspring (PCB180).

Conclusions: High purity PCB180 and PCB52 cause distinct pattern of effects, partly similar to and partly different from those of DL-PCBs. Risk characterization based on the observed liver and thyroid hormone effects and adipose tissue concentrations (this study and human data) suggests a margin of exposure for the adult general human population, which is several orders of magnitude for these individual NDL-PCB congeners.

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