Tees Estuary Seal Study (TESS)

II – Health and Welfare

TEES AND NORTH-EAST HARBOUR SEAL PUP MORBIDITY

Preliminary investigation 2024

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Summary

The TESS trial photo-monitoring of the Tees harbour seal colony during the pupping season 2024 confirmed 22 pups on 10/07/24. Between late July and the end of August 20 pups were reported stranding, in a moribund condition, between north Yorkshire and Northumberland, and a further three during the following month. Because there is no other known pupping colony of harbour seals for a hundred miles to the north and south of the Tees, most of these stranded pups are likely to have been born in the Tees.

Most of the stranded pups were extremely underweight and had tissue necrosis “mouth rot” in the oral area (photo 1). Most of the pups had to be euthanised (or later died) on account of the severity of their infection and irreparable tissue damage. Bacteria cultured and isolated from the mouth rot tissue of five euthanised pups were gram-negative bacteria (*Serratia fonticola* and *Vibrio* spp) in two pups stranding on 02 and 06 August, while *Streptococcus* species in three pups stranding between August 31 and October 04 included *S. phocae* and *S. agalactiae*.

Blubber samples from six pups were found to have moderately high concentrations of PCBs. Comparison of these levels with those from other studies suggested that the concentrations found may have been sufficiently high to be responsible for the pups’ small body size and possibly also for their weakened immune response to infection. Continuing investigation in the 2025 pupping season will be necessary for a better understanding of harbour seal pup morbidity in north-east England at the present time.

Photo: Sally Bunce (2022)

Photo 1. stranded harbour seal pup with oral “mouth rot” extending to face

1. Background – the Tees harbour seal breeding colony, summer 2024

The Tees harbour seal colony pups are born in late June and early July. The Tees Estuary Seal Study (TESS) carried out trial counts using photos and drone videos during June 24–27 and July 7–11 to record the total number of harbour seals and newborn pups visible and to check on pup behaviour and health via photo recording (Photo 2; Tees Estuary Seal Study (TESS I – Tees harbour seal pupping, 2024). On July 10 our maximum pup count was on July 10, with 22 pups confirmed.


Photo 2. Seal Sands seal pupping habitat - Seals hauled out at edge of Seaton Channel (July 2024) (TESS survey 10/07 and 11/07 2024)

Two dead pups were noted on the Seal Sands mudflats. However, observations of live pups did not reveal any problems with young pups stranding alone or showing physical weakness, such as being unable to follow their mother.

2. Harbour seal pup strandings and morbidity reported in north-east England, summer 2024

Local seal pup rescue reports indicated about 23 harbour seal pup strandings between North Yorkshire and Northumberland (20 in late July/Aug, two in Sept, one in early Oct) in 2024. Of 17 of these pups, 15/16 had “mouth rot” or MR (necrotic dermal tissue in the oral area), 12 were euthanised at a vet surgery and five sent for rehabilitation at a seal hospital (one to Hessilhead and four to Tynemouth). The four at Tynemouth Hospital subsequently died; no information is available at present on the pup at Hessilhead.

Further information for eight pups on stranding locations, body weight together with a sample of the MR area and skin/blubber was made available by the attending vets[1] to the TESS team (Table 1). Four of these pups (TS 1, 3, 6, 8) stranded within Tees Bay, one (TS2; photo 3) in North Yorkshire, one in Newcastle (K. Edward’s Bay) and two (TS#s 4&7) in Northumberland.

Pups TS 1, 2 and 3, stranding in the immediate post-weaning period, were all below average normal birth weight of ~11 kg. Pups stranding later in the season were heavier, although still very underweight for their age, with pups TS 4, 7 & 9 weighing less than 13 kg.

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[1] R&P – Robson and Prescott, Morpeth; Beck – Beck vets, Whitby; Medivet, Darlington, M&P – Morris & Plumley, Alnwick.

Photo 3. Pup TS2 (06/08/24). Photos: Sally Bunce

Table 1. Euthanised pup stranding and sampling data

Pup#	date	Location	Vet	Weight (kg)	MR sample	Blubber sample
TS1	02/08	Seaton Carew	R&P	~9 (estimate)	Y	Y
TS2	06/08	Runtswick Bay	Beck	8	Y	Y
TS3	16/08	Greatham, Tees	Medivet	7.3	No MR	I*
TS4	22/08	Cresswell	R&P	12.5	Y**	Y
TS6	31/08	Redcar	Medivet	13.5	Y	I*
TS7	03/09	Boulmer	M&P	12.5	Y**	Y
TS8	29/09	Hartlepool	R&P	15.8	Y	Y
TS9	04/10	Newcastle	R&P	12.4	Y	Y

I* = insufficient sample for analysis; Y** sample location uncertain

Comment. There is no other known breeding colony of harbour seals for 100 miles to the south of the Tees Estuary or for more than a hundred miles to the north. Therefore it seems likely that most of the pups stranding in the summer between north Yorkshire and Northumberland were born in the Tees. Since the total number of reported strandings in this area was approximately equal to the number of live pups counted in the Tees breeding colony during the July pupping period, it seems that the Tees pup post-weaning morbidity in 2024 may have been close to 100%.

3. Mouthrot (MR) pathology investigation by bacterial culture, TESS, 2024

The affected oral MR area was usually on the hard palate – with the exception of pup TS2, for which the necrotised tissue was mainly on the outer area of the lower lip (see photo). Pups were euthanised if the tissue necrosis was considered too severe for potential recovery; pup TS9 was the most severely affected, with the tissue necrosis having caused a complete cavity through the upper palate and the infection continuing through the sinuses to the ear. Seven MR samples were analysed by VPG (Leeds) by bacterial culture. Gram negative bacteria were isolated from the first two samples (*Serratia* from TS#1 & *Vibrio* from TS2) and gram positive bacteria – different species of *Streptococcus* – from the later pups (TS6, 8 & 9; Table 2). No bacteria were isolated from the samples taken from pups TS4 &7 (Table 1); however, this may be explained by most or all of the necrotic tissue having been removed from those pups prior to samples being taken for the TESS study.

Comment

Since a different type of bacteria was isolated from each of five pups (*Serratia* and *Vibrio* each in one pup early in the season and *Streptococcus* species in each of three pups later in the season), it would seem that the pups were being infected by opportunistic bacteria in their environment. The bacteria isolated identified in the mouthrot tissues are all known to be capable of causing tissue necrosis in humans, particularly in individuals with a weakened immune system (Baker-Austin et al., 2024 re *Vibrio*; Roberts et al., 2021; Kunjalwar et al., 2024 and Norman et al., 2021 re *Serratia* species) and *Streptococcus* species in marine mammals as well as in humans (Numberger et al., 2021).

Both *Serratia* and *Vibrio* are waterborne and thrive in warm coastal and brackish waters – such as where newly weaned harbour seal pups are most likely to be feeding on small fish and shrimps. Concern has recently been expressed about the increased prevalence of the pathogenic *Vibrio* species in warmer waters and weather (due to climate change; Archer et al., 2023), with concern expressed with reference to increased prevalence of *Vibrio* infection in UK shellfish sites (Harrison et al., 2022) and increased prevalence of *Serratia* as a clinical pathogen in intensive care human patients (Schwab et al., 2014).

Streptococcus species are gram-positive bacteria which may be susceptible to traditional antibiotics, such as penicillin. However, the gram-negative class of bacteria, such as *Serratia* and *Vibrio*, have an outer membrane which protects them against certain antibiotics such as penicillin, and when their cell wall is disturbed, they release endotoxins. Gram-negative bacteria (but not gram-positive bacteria) may be partially killed by a mixture of hydrogen peroxide and ascorbic acid, which damages their cell walls; antibiotic treatment requires identification of the species and its antibiotic sensitivity. However, since seals (and harbour porpoises) may be subject to infection by antibiotic resistant bacteria (Norman et al., 2021); this is a challenge for MR-infected harbour seal pups taken into rehabilitation centres – particularly in the summer season when gram-negative bacteria may be more prevalent in the coastal environment

The questions remain of why the infections were invariably found in the pups' oral area, especially in the hard palate. One possibility may be that the pups were being infected from bacterial-infected fish or shrimps that they were eating. This hypothesis would be consistent with the observation in 2024 (also in 2023) of Tees pups still being fed by their mothers *not* overtly suffering from oral infection. However, a drone survey in August 20924 revealed a weaned pup hauled out with the colony with evident MR and an underweight yearling with evident MR was observed hauled out at the Tees in mid-July 2023 (Photos 4 and 5). Bacterial tissue necrosis is known to escalate rapidly from the infection site and cause morbidity within a matter of a few days (e.g. Roberts et al., 2021) – this could explain why so many pups stranded in the first half of August, i.e. shortly after weaning.

Photo 4. Two small harbour seals believed to be yearlings at Tees Estuary (Greatham Creek), 15/07/23. The seal on the left appears healthy; the seal on the right appears underweight and with a swollen muzzle (Photo; S. Wilson)

Photo 5. Harbour seal pup at Seal Sands, Tees Estuary (TESS drone survey 04/08/24). Pup appears to have MR.

4. Blubber sample analyses for PCBs (polychlorinated biphenyl compounds), TESS, 2024

Sufficient blubber samples were obtained from six pups (TS 1, 2, 4, 7, 8 &9) for PCB analysis (Table 2). Five of the six pups were extremely emaciated (see Photo 3 of pup TS2), with pup TS8 weighing slightly more. The %lipid in the samples ranged from 4.8% (TS4) to 30.7% (TS9). The actual amount of fat extracted from the sample was dependent partly on the size of the skin area sampled, which was variable.

Table 2. Blubber samples from six euthanised pups analysed for PCB (lipid wt.) content

Pup	Pup wt (kg)	Sample wt*. (g)	Lipid wt. (g)	%lipid
TS1	~9	17.6	4.6	26.1
TS2	8.0	9.8	0.6	6.1
TS4	12.5	41.8	2.0	4.8
TS7	12.5	14.4	1.8	12.5
TS8	15.8	32	2.0	6.3
TS9	12.4	29.8	10.3	30.7

***Each sample included skin (with hair) and associated blubber**

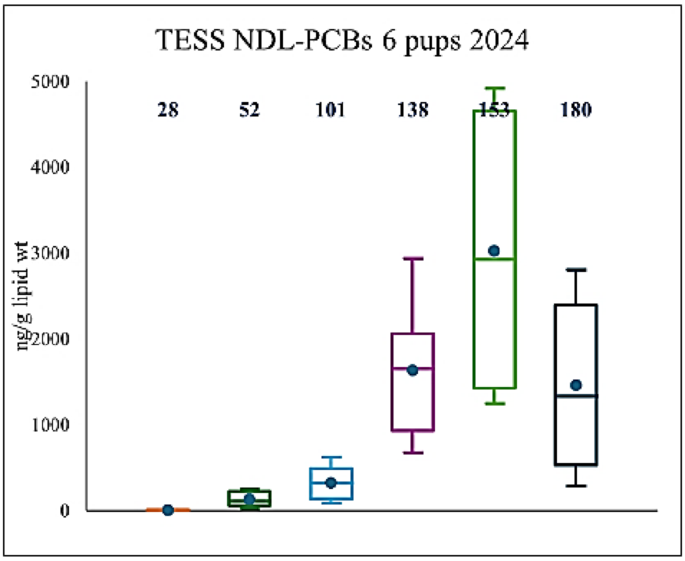
The analyses were carried out at UKAS Veritas lab, Southampton (Karolina Pettit) for 12 (WHO) dioxin-like PCBs (DL-PCBs) and 6 (EC) non-dioxin-like PCBs (Appendix A; see also Appendix B for explanation of DL and NDL PCBs). A summary of the DL and NDL PCB concentrations are given in Table 3. The sum of the 12DL concentrations occurred in trace amounts (<1ppm), whereas the sum of the 6DL concentrations ranged from 2.5 to 11.1 ppm lipid wt. (Table 3). The highest concentrations of both DL and NDL PCBs were in pups TS 1, 2 and 9.

Table 3. Summary of bacterial culture and PCB analyses for seven stranded and euthanised harbour seal pups 2024. PCB concentrations in µg/g (ppm- parts per million lipid wt.)

Pup	Bacterial culture	Σ12DL-PCBs	Σ6NDL-PCBs
TS1	*Serratia fonticola*	0.596	10.521
TS2	*Vibrio* spp.	0.770	11.146
TS4	None isolated	0.167	3.794
TS6	*Streptococcus* spp.	NA	NA
TS7	None isolated	0.125	2.473
TS8	*Streptococcus phocae*	0.118	2.712
TS9	*Streptococcus agalactiae*	0.368	8.957

5. Breakdown of PCB analyses from all results from the six TESS pups, 2024

Of the six (EC) NDL-PCB congeners, #s 138, 153 & 180 made the most contribution to the total concentrations of NDL PCBs (Figure 1-top). Of the 12 DL-PCB congeners, the greatest contributions were made by congeners 105, 118 & 156 (Figure 1-bottom). However, the congeners considered to be the most toxic (#s 77, 126 and 169) were barely in evidence in the TESS samples.



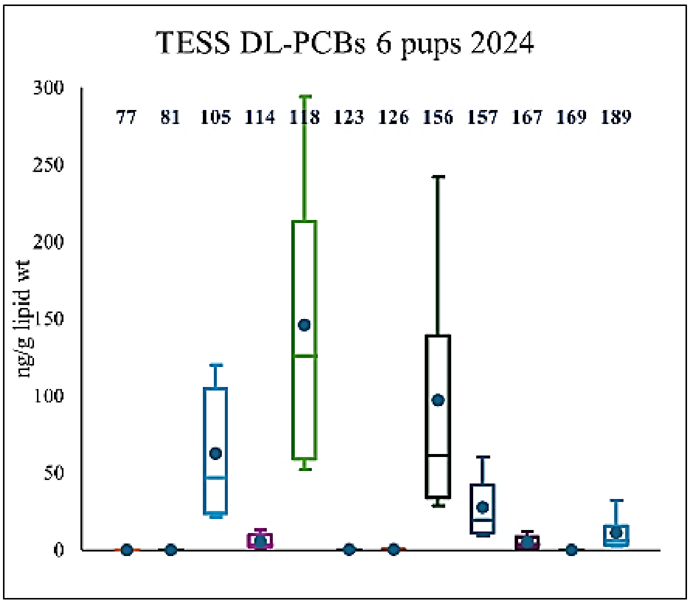


Figure 1. Concentrations of individual DL-PCB and NDL-PCB congeners (ng/g – ppb lipid wt.) in blubber of six harbour seal pups euthanised Aug–Oct 2024. Horizonal line is median, blue dot is average.

Comment

Although the DL-PCBs occur in the seal blubber in only tiny amounts relative to the NDL concentrations, they are nevertheless thought to exert the most toxic effects, including reduced glucose uptake, thymic atrophy and immunotoxicity.

The immunotoxic effect (toxic equivalent concentration, TEC) of each of the DL-PCB congeners is estimated by multiplying by a toxic equivalency factor (TEF). The TECs for each PCB congener are then summed to produce a total toxic equivalency (TEQ). The potential threshold for immunotoxic effect of TEQs calculated from *all* dioxin-like POPs (only PCBs were analysed in the TESS study) is 0.209 ppb (Imaeda et al, 2009). The TEQs for each of the TESS samples from the PCBs alone ranged from 0.02 (TS8) to 0.112 (TS1); other dioxins were not analysed. It seems likely that the toxic effects of the DL-PCB concentrations in the TESS pups may have contributed further to the toxic effects of the NDL PCBs, even if they were not sufficient on their own to result in immunosuppression.

The TESS pups' low body mass (especially TS 1. 2 & 3 in the early post-weaning period) and the low % extractable lipid is not only reminiscent of the 1989-93 underweight pups (Appendix C), but also of the so-called "wasting syndrome", where the presence of POPs, particularly dioxin-like PCBs, may cause rapid energy depletion (reviewed by Robinson et al., 2018).

It is thought that relatively high levels of DL-PCBs increase lipolysis of the blubber layer while the pup is in negative energy balance (as in the immediate post-weaning period while the pup is learning to forage) and exert the greatest negative impact on the leanest pups. Thus the TESS pups, particularly those in the early season, would have been in a negative energy spiral – and likely non-viable, even if they had not become infected with the necrotising bacteria.

6. Estimated total PCBs in the TESS six pups' blubber samples

In the TESS study only 6 NDL PCB congeners (the EC6 markers) were analysed, and therefore the ΣEC6-PCBs is not equivalent to the sum of 25PCBs analysed in some studies (e.g. Williams et al., 2019 in harbour porpoises). However, a simplified analytical method was established in Europe where the 6EC indicator NDL congeners are quantified (as in the present study) and the total ΣPCBs are estimated as five times the sum of the EC6 congeners (Ishikawa et al., 2004). If this calculation is carried out, the estimated total ΣPCBs in the six TESS samples are as shown in Figure 2.

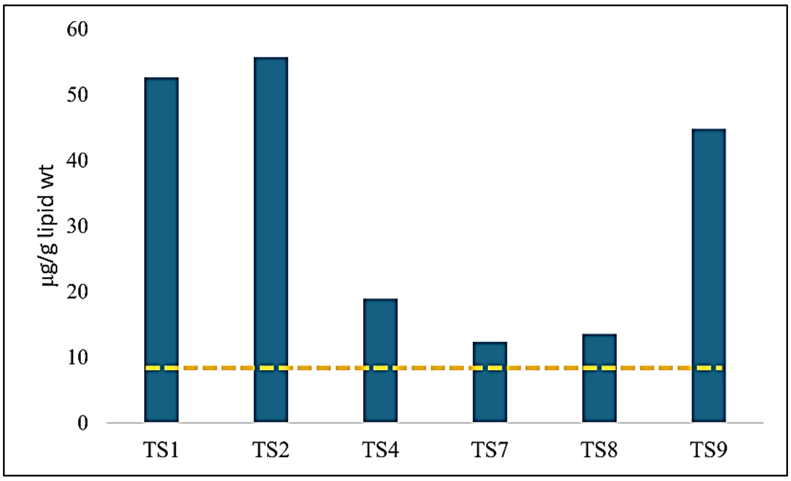


Figure 2. Concentrations (μg/g – ppm) of Total ΣPCBs estimate (ΣEC6x5) in blubber of the six TESS pups (2024). The orange broken line indicates the generally accepted threshold of ΣPCB concentrations (8.7 μg/g - ppm lipid wt.) for immunosuppression risk.

Comment

The generally accepted threshold for likely immunosuppressive effect is ΣPCBs ~8.7 ppm (μg /g) lipid wt. in the liver (Kannan et al., 2000) – although it is not clear how this may relate to ΣPCB concentrations/lipid weight in the blubber layer. It also seems unlikely there is an exact threshold. However, Figure 3 indicates that this hypothetical threshold was exceeded for all the six TESS pups and by 5–6 times for pups TS1, 2 & 9. Although a higher threshold of 17 ppm lipid wt. in blubber was identified as being associated with disease in stranded harbour porpoises (Jepson et al., 2005), it seems likely that there is no precise "cut-off" concentration of total PCBs where immunosuppression begins to take effect in underweight post-weaning seal pups.

A further caveat is that the estimated total PCBs is only estimated by a "rule of thumb" method, which could be refined (Ishikawa et al., 2004). It does not take account of the effects of the 12 DL-PCBs, because the concentrations of these are too low to have much effect on the total sum.

It has been demonstrated (in vitro) that harbour seals are more vulnerable to immunotoxic effects of PCBs than grey seals (Hammond et al., 2005). It is thought that the NDL-PCBs at high concentrations may have a neurotoxic effect, particularly in very young animals (Robinson et al., 2018), possibly resulting in disorientation and abnormal movement patterns (e.g. Giesy & Kannan, 1998; Viluksela et al., 2012). This might partly explain the locations far from the Tees where the TESS pups stranded.

7. Comparison of blubber PCB concentrations between the TESS 2024 and other studies

a. Comparison of blubber PCB concentrations between TESS six post-weaning pups (2024) and suckling grey seal pups on the Isle of May, Scotland (2017).

The order of abundance of the DL and NDL PCBs found in the TESS harbour seal pups is the same as that reported for blubber in grey seal pups in the Isle of May, Scotland (Robinson et al., 2019; Bennett et al., 2021), although the concentrations were very much higher in the TESS pups (see quantitative comparison below; Figure 3).

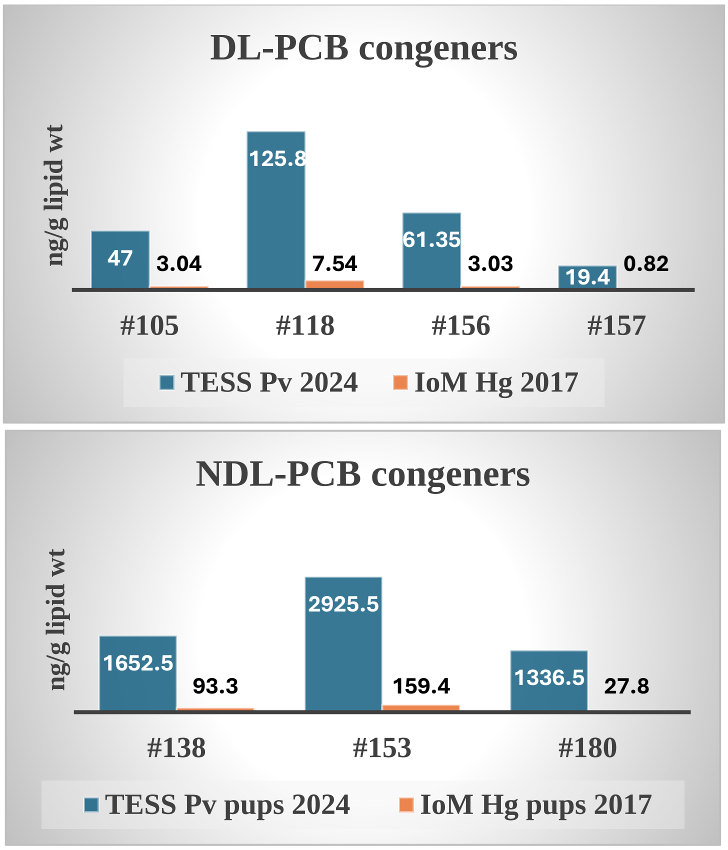


Figure 3. Median concentrations (ng/g – ppb) of the most prevalent PCB congeners in the blubber of six harbour seal (Pv) pups in the TESS study 2024 and in the blubber of grey seal (Hg) pups in the Isle of May (IoM) study 2017 (Bennett et al., 2021).

The PCB concentrations in the blubber of suckling grey seal pups (on the Isle of May, Scotland) were found to have a detectable suppressive effect on pup growth by causing reduction in blubber glucose uptake and affecting the levels of thyroid hormones (Robinson et al., 2018; Bennett et al., 2021). PCBs #105 and #118 may activate thyroid hormone receptors (TR) (Gauger et al., 2007).

Nevertheless the comparison of the DL-PCB congeners between the IoM grey seal pups and the TESS harbour seal pups indicates the concentrations in the TESS pups were approximately 15–23 times higher than in the IoM grey seal pups, with the concentrations of #105 and #118 suggestive of considerable effect on thyroid gland function (Figure 2-top). Comparing the NDL-PCB congeners, the concentrations in the TESS pups were 17–18x higher for congeners #138 and 153, and 48x higher for congener #180 (Figure 2-bottom).

Comment

After weaning, before the pup starts to feed effectively, the fat in the blubber layer is mobilised to supply the pup with energy. The mobilised fat, along with associated PCBs, enters the bloodstream, transporting both fat metabolites and PCBs to body organs, where the PCBs may exert negative effects on cellular activity (Robinson et al., 2018). It is likely that the more lipophilic PCB congeners may become more concentrated in the remaining fat – until the subcutaneous fat layer is almost completely depleted (Louis et al., 2016).

Almost total blubber depletion was the case in the six TESS pups. This may mean that not only the less lipophilic PCBs may have already entered the bloodstream, but likely most or all the congeners present in the metabolised fat may have already been transported around the pups' bodies by the time of blubber sampling from the emaciated, stranded pup.

Therefore, the higher PCB levels in the six post-weaning and emaciated TESS pups than in the apparently healthy grey seal suckling pups on the Isle of May may have a dual possible explanation,

(1) the grey seal pups were healthy, were still suckling when sampled and thus were still in a state of positive energy balance, whereas the emaciated TESS pups were weaned, were in an acute state of negative energy balance, and undoubtedly had PCBs circulating in the blood at the time of stranding and sampling,

and/or

(2) the TESS mothers may have been feeding locally on contaminated fish in Tees Bay, thus providing heavily contaminated milk for their pups, whereas the grey seal mothers in Scotland are likely, in recent years, to have been feeding in less PCB-contaminated areas (Robinson et al., 2019). A sample of shrimps, sprats and other fish species from Tees Bay in November 2024 have now been obtained and will be analysed for PCBs.

b. Comparison of PCB concentrations between TESS six pups 2024 and four pups dying in eastern England during the 1988 PDV epizootic

A comparison of median concentrations of four PCB congeners in our six TESS pups and four harbour seal pups dead during the 1988 phocine distemper epizootic in eastern England (Law et al., 1989) suggests the levels were similar (Figure 4).

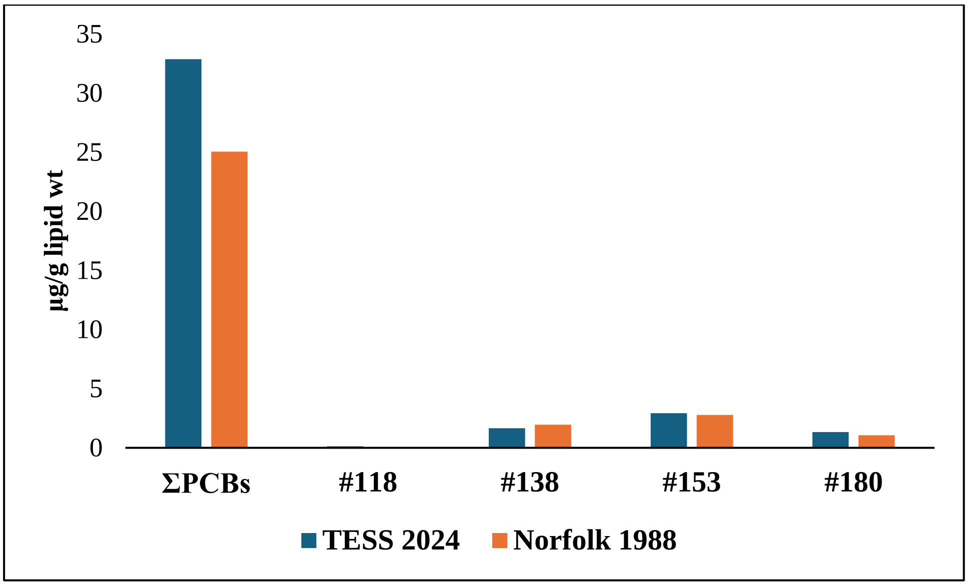


Figure 4. Comparison of median concentrations (lipid wt.) between TESS six pups 2024 and Norfolk four pups 1988 for ΣEC6 PCBs x 5 (TESS), ΣPCBs (Norfolk), DL congener #118 and NDL congeners 138, 153 & 180) (Norfolk data from Law et al., 1989).

c. Comparison of PCB concentrations between TESS six pups 2024 and a study of human mothers and infants in eastern Slovakia

A study of six PCBs in perinatal maternal serum lipid calculated the sum of six PCB congeners (DL-PCBs #s 118 & 156 and NDL-PCBs #s 138, 153, 170 & 180. The range of concentrations was 190–1170 ng/g serum lipid (Park et al., 2008).

The TESS pup analysis included five of these PCBs (#170 was not analysed). However the range of the sum of the five congeners in the pups was 2442–10804 ng/g lipid and median & average concentrations of 6102 & 6374 ng/g lipid, i.e. approx. 9–12 x higher than the human maternal serum lipid concentrations.

The human serum concentrations were found to be associated with up to a 7% reduction in the newborn infant's thymic index (Park et al., 2008). This reduction was considered to affect the differentiation and maturation of T lymphocytes and thus affect the infant's immunologic development. These authors also suggested the thymus reduction may have been responsible for births to be a week premature.

Comment: This comparison between human perinatal maternal serum lipid PCBs and harbour seal pup post-natal blubber PCBs is evidently not comparing like with like, since in our TESS pups, blubber PCBs will have been mobilised and transported in the blood at unknown serum concentrations. Nevertheless, it is possible that PCB concentrations per lipid weight *may* be comparable whether sampled in blubber, liver or serum. As with the human infants in the Slovakia study, it is likely that most of the TESS pups' PCB burden came from their mothers' milk (Debiér et al., 2003), and also that they were exposed prenatally to maternal serum PCBs.

8. Interim conclusions

Our preliminary 2024 investigation suggests that the underlying cause of the high morbidity levels in weaned pups in north-east England in 2024 may be poor post-natal growth combined with a weakened immune system, caused by a PCB cocktail ingested in the mother's milk (e.g. Debier et al., 2003). The pups TS 1, 2, 3 & 6, stranding in the early postweaning period (02–16 Aug) were all well below normal birth weight. Such emaciated pups are then highly susceptible to lethal infections by potentially tissue necrotising bacteria.

The possible culpability of PCBs in the seal pup morbidity was suspected from the Tees seal study carried out in 1989–1993, when the three pups that were born died soon after birth and were found to be very underweight with high levels of PCBs in their blubber; pups dying later during their first year also had relatively high levels (Appendix C; Wilson, 2001; 2022). However, the analysis at that time only gave a sum of total PCBs, and therefore we are unable to compare the levels of the individual DL and NDL congeners.

The Tees pup morbidity is occurring mainly in the immediate postweaning period. Two principal factors may have combined to make these pups non-viable – their low body weight and hence physical weakness, and their mouth-rot bacterial infection. Both of these principal factors are likely to have an underlying cause of the relatively high PCB contamination of their body tissues, indicated by the blubber analyses for PCBs. PCBs are known to cause poor prenatal and postnatal growth of the newborn seal pup, and they are also known to cause immunosuppression in harbour seals and other marine mammals. The PCB findings do not rule out contributions to the from other pollutants but may be enough on their own to account for the extraordinarily high postweaning pup mortality, seemingly of most of the Tees 2024 cohort of pups.

9. Recommendations

1. Further samples for bacterial culture and PCB analyses are needed from pups euthanised with MR in late July and first half of August. This is to assess the prevalence of gram-negative bacteria such as *Serratia* and *Vibrio* in summer while the coastal waters are relatively warm (In 2024 we were able to obtain only two samples from pups stranding in the first half of August, although there were about 13 others during that period). More samples later in the season (late August–October) are also needed.
2. A swab for bacterial culture should be taken from the infected tissue area of any pup stranding with relatively mild MR and considered a candidate for rehab. The bacterial culture result will inform on the bacterial type and enable veterinary advice to the rehab centre on the best possible course of treatment. Gram negative bacteria such as *Serratia* and *Vibrio* may be resistant to some antibiotics and may be susceptible to peroxide mouth or tissue wash as supplemental treatment.
3. A new set of samples of fish and shrimps should be taken for bacterial and PCB analyses. Samples for bacterial analysis should be taken in coastal waters in both the summer and in the autumn. Investigation of PCB concentrations in fish, crustaceans and molluscs in the Tees estuary region in 1990 revealed high PCB levels, with especially high levels in herring, saithe and flounder liver and in brown shrimp (Appendix D; Wilson, 2001; 2022). To our knowledge, PCB levels in dead seal pups, fish and shrimps from the Tees have not been investigated since that time. Fish and shrimp samples taken recently from known points in Tees Bay in November 2024 are awaiting PCB analyses.
4. Although it is not definitely known if the onset of pup morbidity with low body weight and MR coincides with renewed deep dredging of the River Tees, it is a possibility that historic deposits are being dredged up and returned to circulation in the surface sediments and thence through the Tees food chain to seals. Although potential mitigation may already be too late, with much dredge material having already been dumped offshore in Tees Bay during the past few years, the possibilities and technology for extracting dredged material without spillage and depositing it in lined landfill sites must be explored urgently rather than the dredge being redistributed offshore, where it may be affecting the food chain for crustaceans, fish and marine mammals.

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Appendix A. Summary of Analysis of PCB-WHOs and PCB EC6 in seal blubber samples (Veritas Laboratory Services)

1. Sample methodology

A layer of blubber was separated from the seal skin and cut into small pieces. The pieces were placed into clean, new glassware. A 50 ml mixture of 50:50 dichloromethane (DCM) and diethyl ether (DE) was added to the blubber, then macerated to ensure homogeneity. The mixture was sonicated for 20 minutes, after which the extract was filtered through sodium sulphate to remove any water. This sonication and filtration process was repeated twice. The extract was then concentrated until there was no further loss in sample weight.

After determining the lipid content, 2 grams of fat (when available) were spiked with isotope-labelled extraction standards, then purified using an automated clean-up system (Miura) with silica, alumina, and carbon columns. The extract was concentrated to 20 μL, and isotope labelled recovery standards were added. Quality control samples, including blanks and spiked fat samples, were prepared and analysed alongside the blubber samples.

2. Analysis

The analysis of WHO-PCBs and WHO-EC6 is performed on the newest Agilent gas chromatography-mass spectrometry (GCMS/MS). A DB-5MS UI column is used to analysed PCBs to ensure the separation of closely eluting congeners.

3. Analytical Parameters

Polychlorinated Biphenyls
PCB-WHOs PCB EC6
PCB 77 (3,3',4,4'-TeCB)
PCB 81 (3,4,4',5-TeCB)
PCB 105 (2,3,3',4,4'-PeCB)
PCB 114 (2,3,4,4',5-PeCB)
PCB 118 (2,3',4,4',5-PeCB)
PCB 123 (2',3,4,4',5-PeCB)
PCB 126 (3,3',4,4',5-PeCB)
PCB 156 (2,3,3,4,4',5-HxCB)
PCB 157 (2,3,3',4,4',5'-HxCB)
PCB 167 (2,3',4,4',5,5'-HxCB)
PCB 169 (3,3',4,4',5,5'-HxCB)
PCB 189 (2,3,3',4,4',5,5'-HpCB)

PCB 28 (2,4,4'-TrCB)
PCB 52 (2,2',5,5'-TeCB)
PCB 101 (2,2',4,5,5'-PeCB)
PCB 138 (2,2',3,4,4',5'-HxCB)
PCB 153 (2,2’,4,4’,5,5-HxCB)
PCB 180 (2,2’,3,4,4,5,5’-HpCB)

Appendix B. Polychlorinated biphenyls (PCBs)

Information below from *Greenfacts* https://www.greenfacts.org/glossary/pqrs/pcbs-polychlorinated-biphenyls.htm

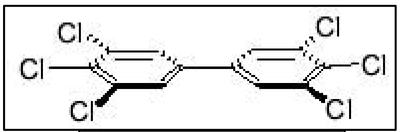
Definition of PCBs. Polychlorinated biphenyls (PCBs) are a family of 209 congeners of structurally similar organic chemicals, ranging from oily liquids to waxy solids. There are 12 PCBs that are dioxin-like and can similarly be toxic and non-toxic.

PCBs are synthetic and produced either as a singular congener, as a homogeneous group or as a mixture. They are non-flammable, stable, have a high boiling point and exhibit electrical insulating properties. As such, PCBs have been used as coolants and lubricants in transformers and other electrical equipment, as hydraulic fluids, and as plasticizers, pigments, dyes and carbonless copy paper ink. They are also generated and released into the environment as waste byproducts of chemical manufacturing and incineration.

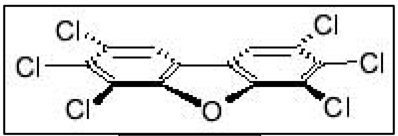
Structure of different PCB types. Each PCB molecule contains two phenyl rings. A phenyl ring is a ring of 6 carbon atoms to which hydrogen atoms are attached. In PCBs, one or several chlorine atoms replace some of these hydrogen atoms. The two rings in a PCB molecule can rotate around the bond connecting them.

Depending on where the chlorine atoms are located, the two rings of a specific PCB will either:

- lie approximately in the same plane (coplanar or “dioxin-like” (DL) PCBs) or

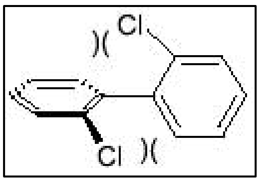


Dioxin-like PCBs



Dioxins

- lie in different, more perpendicular planes (non-planar, non dioxin-like (NDL) PCBs).



Non-Dioxin-like PCBs

Coplanar DL-PCBs are considered to be most toxic, based on combined health effects considerations.

(PCB description, explanation and diagrams from *Greenfacts*)

Appendix C. Tees seals - toxicology results 1989–1993 (from Tees Seals report 1994).

Table 6. Concentrations of contaminant organochlorines and metals in seals dying at or near Teesmouth.

SEALS	Date (mo/yr)	PCB ppm	DDT ppm	% HEL	Zn ppm	Cu ppm	Hg ppm	Source of analysis
newborn local C pups								
5-d pup ♂	7/89	114	9	29	60	26	1	MAFF
1-d pup ♀	7/91	6	2	44	112	8	0.5	AES
4-d pup ♂	6/93	41	8	15	68	11	0.8	AES
transient C pups								
5-m pup ♂	12/88	19	3	74	39	11	1	MAFF
3-m pup ♂	10/90	28[1]	2[1]	68	31[2]	8[2]	0.2[2]	[1]M-Scan/ [2]ICI
4-m pup ♀	11/90	13[1]	1[1]	78	44[2]	14[2]	0.2[2]	[1]M-Scan/ [2]ICI
transient G pup								
1-m pup ♀	12/88	39	7	61	60	4	0.3	MAFF
adult seals								
Adult C ♀	11/93	7	1	75	23	1	28	AES
Adult G ♂	4/93	10	3	86	197/ 50	50/ 17	7/ 4	AES/ MAFF
Adult G ♂	5/93				53/ 55	24/ 18	372* /193	AES/ MAFF

C = common seal; G = grey seal; PCB & DDT concentrations in blubber given in ppm (mg/kg or parts per million) lipid weight; %HEL = % hexane extractable lipid; metal concentrations in liver given in ppm wet weight. The origin of the seals other than the 3 newborn Tees pups is not known. * The Hg level in the liver from this seal was initially found to be 2541 ppm wet weight, but this extraordinary level was not confirmed on reanalysis by MAFF or by AES a month later. The liver sample had shrunk and lost half its original water content during storage. If the mercury had been present as dimethylmercury, then loss could have occurred as this is a volatile liquid.

Appendix D. Tees fish & shrimp toxicology analysis 1990

Table 7. Tees Seal Research Programme survey of fish and invertebrates in the Tees (Aug-Sep 1990)

Location	species	*organochlorines* PCB	DDT	*metals* Cu	Zn	Cd	Pb	Cr
		mg/kg lipid wt.		mg/kg wet wt.				
Estuary	whiting (liver)	4	0.2	3	11	0.1	ND	0.2
	flounder (liver)	4	0.5	10	39	0.2	ND	0.3
	saithe (liver)	7	0.6	2	17	0.1	1.6	0.4
	herring (liver)	10	0.6	1	18	0.1	1.0	0.2
very small ('0' class) whole fish								
	whiting	1	0.1	1	15	0.1	ND	0.4
	saithe	1	0.1	3	8	0.1	ND	0.1
	sprat	4	0.4	2	17	0.1	0.7	0.2
invertebrates								
	shore crab (soft parts)	4	0.4	51	26	0.1	1.5	1.2
	brown shrimp	18	0.5	7	24	0.1	0.9	0.2
	mussel (S.S. Peninsula)	3	0.3	3	92	0.4	4.9	4.2
	(S.S. south wall)	4	0.3	5	48	0.8	1.9	3.4
	winkle (S.S. south wall)	9	0.1	43	25	0.5	2.1	2.9
River	flounder (liver)	16	1.5	15	56	0.1	ND	ND

ND = undetectable concentration; S.S. = Seal Sands