

Report of the IWC Pollution 2020 Steering Group Meeting

Sea Mammal Research Unit, Scottish Oceans Institute, University of St Andrews

Tuesday 25th - Friday 28th March 2014

1. Introductory items

1.1 Introduction of participants

The Steering Group members were introduced and a list is shown in Annex A.

1.2 Appointment of Rapporteur

Joanna Kershaw from the Sea Mammal Research Unit was appointed rapporteur.

2. Presentation of Pollution 2000+ Phase II and III Modelling Work

The last 4 years has seen the completion of Phases II and III of the Pollution 2000+ initiative that has included the finalisation of an individual based model that can be used to investigate the effects of pollution (particularly polychlorinated biphenyl or PCB) exposure on cetacean *populations*. The group discussed the applicability of the model as it currently stands and how it could be improved (see also section 5.1).

1. The model doesn't include the potential effects of PCBs on the immune system of the developing foetus. However, it is unlikely that there are any data to assist in quantifying any effects *in utero*. Thus, while including this effect would be desirable, it is not currently possible so it was recommended that this be mentioned in the explanation of the model.
2. The individual based model has been used to study the effects of pollutants on humpback whales in the Gulf of Maine and bottlenose dolphin populations (Hall et al., 2012; Hall et al., 2011) but it could also be used to model the well-studied population killer whales off the coast of British Columbia. There are data on vital rates, population size and blubber PCB levels. Another example would be Beluga, as again there are data on blubber PCB levels in these animals, and possibly also vital rates.
3. The group then discussed the drawback that the accumulation of PCBs in the females is set at a constant annual rate. As annual accumulation is likely to change over time, it would be better to reflect the gradual decrease in environmental PCBs. There are a number of longer term datasets (e.g. data for the killer whales in Puget Sound and 2005-2010 data for the Sarasota Bay bottlenose dolphins) that could assist in determining the rate of decrease over decades. In addition, the vital rates used to parameterise both the dolphin and the humpback model may be outdated so efforts to update those where possible would be helpful.
4. One of the major sources of uncertainty in the model are the parameters that control the offloading of PCBs from mothers to their calves; both *in utero* transfer and transfer during lactation. Better data on maternal transfer would greatly improve the model. More data from captive studies on bottlenose dolphins and from the Navy dolphins through longitudinal studies on mothers and their calves might be available in future. For example, the humpback model for the Gulf of Maine does not capture the high PCB levels in the juveniles and then the dramatic decrease when they become sub-adults. This could be because:
 - the maternal transfer of contaminants is inaccurate (as discussed above).
 - growth is not included in the model. The concentration of PCBs in the blubber should decrease as an animal grows over the first few years of its life as its volume of blubber increases. It may be possible to build a separate model for the effects of growth on blubber PCB concentrations over the first few years of an individual's life and then embed this into the full model. The effects of changing condition on PCB blubber levels has been studied in California sea lions so this could be used as a model (Hall et al., 2008).

5. Based on the data from bottlenose dolphins, the relationship between the immune function ConA stimulation index and PCB concentration in the blubber is assumed to be linear. However, it is possible that this is not a linear relationship, but instead, there is a threshold PCB concentration above which the ConA stimulation index starts to drop. This might be clarified by additional *in vitro* data from ConA and PCB experiments. However, whether *in vitro* studies are representative of what is happening *in vivo* is uncertain.
6. Currently, there is no uncertainty incorporated into the model around the relationship between immune function and reduced survival probability. When uncertainty is included, the confidence intervals around the population projections are likely to be very large. However, it would be worth including uncertainty into this relationship using the standard errors presented in the National Toxicology Programme studies.

2.1 The Physiologically Based Toxicokinetic (PBTk) model

In Phase III the individual based model framework included a Physiologically Based Toxicokinetic (PBTk) model to investigate the possibility of including oral dose data as dose-response relationships (Hall et al., 2013). However, there are many uncertainties based on physiological assumptions:

1. Lung morphology and physiology of cetaceans is different to terrestrial mammals which makes the PCB transfer coefficients used in the compartmental model inaccurate.
2. It may be necessary to change the whole compartmental model itself to more accurately reflect cetacean physiology. This could be done by validating the compartmental model first :
 - a. Use PCB concentration data from dead animals to investigate the relative proportions of the contaminant load in the different tissues
 - b. Use PCB concentration data from live animals to investigate the transfer of contaminants in the blood, blubber and urine to estimate partition coefficients.
3. These data may assist in adjusting the coefficients of the compartmental model. Better estimates for cardiac output for dolphins were provided at the meeting (Sommer et al., 1968). However, tachycardia and bradycardia associated with the dive response may complicate the use of a single cardiac volume measurement.
4. There is a lack of metabolic-PCB specific data with which to parameterise the model. The model requires as an input, an estimate of the rate at which metabolic breakdown occurs in the liver and for PCBs this appears to be available for only one congener.
5. Air exposure to different volatile contaminants also produces uncertainty in the modelling process. PAH data for cetaceans may be available for lungs, liver, blood and urine, but the timing of exposure and the dose of exposure are needed.

There was additional discussion about the next steps in the refinement of the model:

1. To investigate how well the oral dose, PBTk model is performing, compare the estimated blubber PCB concentrations from the model with PCB concentrations in the male bottlenose dolphins from Sarasota Bay. More specific diet data from just the Sarasota dolphins may also be available if required.
2. Look specifically at repeated samples from known individuals over their life time to get a better idea of accumulation of PCBs even if it is just in a few individuals. Collection of repeat plasma samples from some US east coast bottlenose dolphins with associated survivorship data obtained during capture-release studies would be very useful. In addition, more data may be available from the Sarasota Bay dolphins.

These suggestions will be taken forward in further refinements of the model.

3. Demonstration of Web-Based Model Interface

A demonstration of the web-based model interface for the individual based model was shown to the Steering Group. Further improvements were also discussed and the following adjustments to the model for the web-based version were agreed upon -

1. Currently the annual exposure rate to pathogens is set at 5% of the population so include a function to allow this to be changed to reflect a less virulent pathogen and /or the occurrence of epidemics. The pathogen exposure level could be increased by a set amount in a particular time period, and then an inter-epidemic interval can be set for the population
2. Use the two different immune response relationships from the National Toxicology Program data so that a virulent and a less virulent pathogen could be modelled.
3. On the website, it needs to be made clear that the model relies on data from a laboratory model species, the mink, and is not based on data from cetaceans because these data are lacking. Therefore, it needs to be made clear that the model should be used as a comparative *tool*.
4. As mentioned earlier, the annual accumulation of PCBs is currently constant so change this to reflect a decrease in environmental PCBs over time. In addition, episodic exposure to contaminants may also be a useful addition to the model, e.g. have the ability to run the model for a number of 'clean' years followed by the introduction of a contaminant at high levels that then increase over time.
5. Currently, the data for the each population simulation can be downloaded and then reopened in an excel spreadsheet. It would be helpful to include another tab where these saved data can then be reopened and modelled to show the differences between different population simulations using different starting parameters.
6. Currently, fecundity is set as a single parameter. For some populations, age-specific fecundity is known and could be incorporated into population simulations. Therefore, it would be good to be able to choose age-specific fecundity parameters if the data was available
7. General additions to the web-based model were also suggested, including making it possible for researchers to upload their own dose-response data or include their own dose-response function parameters in the model instead of using the default one. In addition a brief explanation of how the models were put together using what data etc. with references to the IWC reports and explanations of how to use the site and interpret the plots.

There was also a general agreement that gaps in the data for modelling population consequences of exposure to pollutants and contaminants need to be identified, and therefore what data would be most useful, and should be collected from future episodic events like oil spills. So whilst for many contaminants it will not be possible to get dose-response relationships, we should be able to get better data every time an event occurs.

4. Microplastics Review

A general presentation was given on the major points arising from the review of the potential impacts of microplastics on cetaceans currently being completed for Pollution 2020.

A number of questions were raised by the Steering Group members:

1. As nanoparticles can diffuse through cell membranes by passive transport, based on the evidence to date, is this a potential problem in cetaceans?
2. Based on necropsies, what evidence should be looked for to assess if microplastics are a problem in cetaceans?
3. The EU Marine Strategy Framework Directive target that there should be "No population level effects by 2020..." was felt to be pretty unrealistic. How would population level impacts be investigated? Perhaps one way might be that because microplastics can concentrate PCBs and other POPs, correlating PCB exposure with microplastics followed by a population level assessment of the additional impact from the concentrating effect might be possible. But it would be extremely difficult to disentangle additional effects due to microplastics exposure.
4. Are microplastics inhaled at the surface? Is there any evidence of this? Could there be effects of inhalation on lung tissues as well as effects on the digestive tract from ingestion?

Dr Cristina Fossi, who was unable to attend the meeting in person, then gave a webinar presentation to the Steering Group on the threat of microplastics in Mediterranean fin whales – a case study. The

group were very grateful to Cristina for giving the presentation. A number of questions arose from the presentation:

1. The MEHP concentrations measured in fin whale blubber are assumed to be indicative of microplastic exposure but could there be other sources of phthalates in the marine environment that are not associated with microplastics? Phthalates are widely distributed in coastal and fresh water ecosystems and are commonly associated with sewage effluent, but no data are available so far on the levels of phthalates on pelagic environments.
2. Preliminary data on MEHP in samples of *Euphausia krohni* (a prey of fin whales) collected in the Mediterranean Sea reported high concentrations of this contaminant ranging from 8.35 to 51.14 ng/g. These results suggested that plastic derivatives also occur in planktonic species (potentially exposed to microplastics) inhabiting the water column (Fossi et al 2012).

Thus one approach to determine whether MEHP is indicative of microplastic exposure, or is indicative of exposure to polluted sewage waters in general, would be to measure another pollutant that is unrelated to microplastic exposure at the same time as the phthalates e.g. caffeine. Therefore, the absence of other markers of sewage exposure would help confirm if blubber MEHP concentrations were indeed indicative of microplastic exposure.

3. There is a need to investigate microplastics in gastro-intestinal tract of stranded animals and grey whales would be of particular interest as a study species as they filter the sediment (an environmental compartment where microplastics may accumulate) as they feed.

The group then discussed the metabolism of phthalates.

1. DEHP is very quickly metabolised to MEHP – between hours and days. It may be useful to look at other metabolites from compounds that have longer half-lives.
2. Metabolites may indicate chronic low level exposure to these compounds because the initial doses are unknown.
3. One matrix in which to measure these metabolites could be in the bile because the half-life is so short and there is likely to be a mixture of compounds (examples of studies in fish (Qu et al., 2014). However, the steering group recognized that collecting bile samples from cetaceans is difficult, as the whales do not have gall bladders.

The Steering Group suggested that other matrices could also be investigated. Fin whale faeces samples have been analysed by Cristina's group in which they detected organochlorines (OCs), flame retardants (PBFRs) and also porphyrins (positively correlated with PCBs concentrations) but not microplastics.

There was also some discussion regarding the estrogen receptor gene expression study in fin whale presented in the webinar:

4. Upregulation of the estrogen receptor in male fin whales has been documented and correlated with contaminant load (OCs and phthalates). Integument biopsies (epidermis, dermis and blubber) were analyzed for the detection of estrogens receptor expression using real time PCR. Whether the receptors are being expressed in the adipocytes or epidermal cells requires further investigation.
5. Male striped dolphins from the Mediterranean also showed increased estrogen receptor gene expression. Striped dolphins are likely to be less exposed to microplastics than the fin whales, so this altered gene expression was not likely to be a result of phthalate (indicating microplastic) exposure alone, but rather a potentially synergistic mix of contaminants in the food chain (including OCs and PBFRs, Campani et al., 2013). There is thus a mixture of contaminants in the food chain and in the water that have the potential to cause endocrine disruption across a range of species not exclusively due to exposure to phthalates from microplastics. It was agreed that it would be very difficult to establish a cause and effect relationship in the striped dolphins because they are exposed to a mixture of pollutants with

endocrine disrupting potential. In line with the endocrine disruptive nature of these pollutants, it would be interesting to investigate spermatogenesis in males with elevated estrogen receptor gene expression. However, this would require freshly dead males in the correct position to get the samples. If possible, qPCR could be used to investigate steroidogenesis gene expression which might be used to identify earlier effects of contaminant exposure.

4.1 Effects of microplastic leachates

The Steering Group then discussed the issues surrounding the potential for widespread impacts of microplastic leachates on cetaceans.

1. Current evidence from fin whales demonstrates phthalate exposure, but that the source of exposure is definitely microplastics requires further research. There is a need to understand the source of exposure, for example to distinguish between untreated sewage sources and direct leaching from microplastics and what the origin of contaminants in the pelagic zone is likely to be.
2. More investigation into the toxic effects of phthalates is required to establish how important the concentrations found in the fin whales are. It is possible that the whales are experiencing high exposures to these contaminants, but current methods do not allow us to detect this because the half-life is so short. It may be that blubber levels are not reflective of overall exposure. The metabolism of these chemicals and their pathway through the body needs to be considered to find the best matrix in which to measure the parent compounds and their metabolites so perhaps bile and faeces would be good indicators for exposure? It might be possible to collect faeces in the field.
3. The current evidence for potential effects of phthalate exposure in fin whales in terms of altered estrogen receptor expression and CYP expression is intriguing, but further studies are needed to confirm this initial finding.
4. The direct and indirect effects of microplastic exposure need to be better defined and understood.
 - a. Direct effects include mechanical/particulate problems and leaching of toxins
 - b. Indirect effects include the problems associated with adsorbed contaminants (see section below).
5. If possible, cetaceans in the regions where microplastics are likely to accumulate should be studied because if there are adverse effects of exposure, they are most likely to be seen in these areas.
6. Whilst there has been some progress in investigating the impact of microplastics in cetaceans, there is a lack of evidence that plastic leachates are, or could be a particular problem. At present, the impact of other pollutants found at higher levels is taking a higher priority.

4.2 Effects of adsorbed contaminants

1. Based on evidence of adsorbed contaminants onto plastic pellets, this may be a more significant problem for cetacean health than leachates. The potential for a concentrating effect of POPs on the microplastic pellets could increase the exposure to these contaminants while, overall, environmental levels appear to be decreasing.
2. There is a need to identify where the risks are greatest, and to which species. For example, PCBs in plastic pellets around the world (e.g. from the International Pellet Watch study <http://www.pelletwatch.org/>) indicates where exposure may be at its highest coupled with estimates of the abundance of microplastics in the water column around the world. An investigation of the feeding strategies of cetaceans may also determine which species may be most at risk. Therefore, based on these three factors, areas and species of research priority could be identified.

3. In order to assess exposure, we need to quantify what goes in (stomach contents) and what comes out (faeces). Therefore stomach contents analysis at necropsy could assist if PCB concentrations in the stomach were related to the quantity of microplastics.
4. In terms of surveillance, which strandings schemes are looking for microplastics in the stomachs of stranded cetaceans (both macro and microplastics)? A standardised method of assessing microplastic and macroplastic ingestion in necropsies is needed to better understand marine debris ingestion in general. In addition, faeces sample collection from live animals should be a priority.
5. A feeding study on pinnipeds would be useful in which levels of PCBs adsorbed onto micropellets by batch are measured, fed to seals then re-measured on the excreted pellets to determine what proportion is retained by the animal. PCBs could be measured in the faeces before and after to estimate a mass balance and determine how much the ingestion of microplastics contributes to PCB burden.
6. An *in vitro* study of use would be to investigate the effects on steroidogenesis in the gonads to establish potential effects on reproduction. *In vivo* studies in mice and fish would also be useful to establish reproductive effects as a result of endocrine disruption. Currently all the published laboratory studies investigating effects have looked no higher than the invertebrates.
7. Need to better assess the specificity of biomarkers to microplastic exposure.
 - a. This would help better establish a cause and effect relationship.
 - b. Using a fresh carcass, investigate the potential biomarkers in the 'target / ideal' organ and then the same biomarker in the blubber. This would help establish the representativeness of blubber biopsies for measuring biomarkers.
8. POPs in microplastics could be an issue.
 - a. Fundamental research using model species is needed to better evaluate whether it could be a problem in cetaceans.
 - b. Therefore, at this stage there is still a limited understanding of the effects of microplastics on higher vertebrates. More data are needed from model species (e.g. Mediterranean fin whale, grey whale, right whale) and the degree of exposure to cetaceans needs to be better characterised.

5.0 Contaminants of concern

At the 2010 Pollution 2000+ Steering Group meeting in California it was concluded that prioritisation regarding current contaminants of concern for cetaceans should be based on a fixed set of criteria. A literature review of pollutant data currently available for cetaceans was carried out following the meeting and a prioritisation questionnaire for experts to complete was compiled. The Steering Group felt that it would be important to continue and complete this task and contacted Dr Stephanie Venn Watson regarding this exercise. The Steering Group collectively completed the questionnaire. Their findings and priorities will then be compared with those obtained from an additional group of marine mammal toxicological experts.

Additional steps in the prioritisation process were discussed:

1. Consult the AMAP reports as that could be helpful for feeding into biomagnification studies.
2. Consult TOXNET for a synthesis of laboratory studies on the toxicity of different pollutants and potential exposure pathways.
3. Review all information on any dose-response relationships between contaminants (focus on the ones that are found in the marine environment) and physiological endpoints. By setting up a database of all the papers published on these relationships in any model species, it will

make it easier to find information on specific chemicals that can be used for future modelling. This database could be updated every 5 years to incorporate new information on different chemicals.

4. Based on the available data, the criteria used to decide the importance of future research into specific chemicals needs to be established. For example, is there any evidence for:
 - a. Potential impact on cetaceans i.e. is there evidence in other mammalian model species that a particular pollutant may have an impact on cetacean health?
 - b. Potential to carry out risk assessments i.e. in practical terms, is there enough information about a certain pollutant to be able to feed in to risk assessments for cetaceans? The available data for dose-response relationships for certain pollutants will dictate which ones can be assessed.
5. If modelling did reveal a population level impact on cetaceans, is there the potential for any mitigation measures to be put in place?
6. Some pollutants are not routinely screened for, but they should be investigated, so work on newer pollutants should be encouraged, e.g. methoxychlor, chlorinated paraffins and musks, current use pesticides and pharmaceuticals, brominated flame retardants. We also need to consider inhalation routes of exposure for PAHs and particulate matter that could cause lung injury.
7. Investigate the potential of using other rapid, inexpensive tests to measure contaminants, e.g. ELISAs for PCBs have been used in bird work (Summers et al., 2010). As it is so expensive to measure contaminants now, new cheaper methods are required for the screening of animals for the newer contaminants that we know very little about.

5.1 Modification of the risk assessment model in future for additional contaminants and endpoints of concern

The Steering Group then returned to discuss how the current framework could be modified in future to include other contaminants and endpoints of concern.

1. Improve the individual based model to reflect changing environment by capturing the effects of different / multiple pollutants and changing pathogen exposure. However, the challenge is how to add multiple contaminants to the same population e.g. as PCBs decrease, PBDEs (or other contaminants) may be increasing? Therefore, incorporate some sort of trade-off between different chemicals as their concentrations are changing in the environment?
2. Incorporate changing pathogen exposure as a result of climate change which may be allowing animals and their pathogens to move into new environments and therefore affect species that were not previously at risk?
3. Investigate the effects of PCBs on the thyroid gland and other sublethal impacts. Quantification requires data from model species but if they are currently available (see dose-response database discussion above), it would be possible to investigate the potential for population level impacts of thyroid disruption and then decide whether it is worth investing more time and effort into this thyroid disruption research in cetaceans. For example reduction in thyroid hormones may lead to a reduction in expression of reproductive hormones which could impact fecundity. Reduction in thyroid hormones also leads to reduced growth and possible reduced early survival probabilities. *Therefore, use the individual based model as a tool to decide where to focus future research efforts.* This would be critical from a management perspective as recommendations could then be made to reduce water pollution from a particular contaminant that appears to be having the greatest effects and may threaten populations. *This will move us forward beyond measuring levels of pollutants in the animals to gathering appropriate data for risk assessments.*

6.0 Biomarkers

The Steering Group discussed the issue of the utility of biomarkers (either of exposure, or of response or ideally of both) in cetacean pollution research. This field has developed widely over the past decade but some consolidation of those that are of real utility because they are specific, sensitive and

reliable. For example, a summary needs to be produced to summarise those of real utility (a suggestion is shown in the table).

			EFFECTS	
		Expression Levels / Concentrations	Reduced Reproduction	Reduced Resilience
Biomarkers that are currently used	Biomarker a			
	Biomarker b			
	Biomarker c			
	Etc...			
Biomarkers that are under development	Biomarker 1			
	Biomarker 2			
	Biomarker 3			
	Etc...			

There needs to be a standardisation of measurement methods and cross-validation studies to establish how useful particular biomarkers are and the use of a range of biomarkers should be investigated. Microarrays could be used more widely and the applicability of hypothalamic-pituitary axis hormones as biomarkers need more validation. Species-specific susceptibility needs to be considered.

Biomarkers need to be identified as tissue specific, or applicable to the whole organism, e.g. CYPs may be less useful than originally thought as they are tissue specific (most useful in the liver) and therefore blubber / skin measurements of CYP gene expression appear, from the results of some studies, to be unrepresentative. More information on the degree of measurement error and intra-assay variability associated with particular analytical methods for determining CYP activity and expression is needed. PAH-DNA adducts may be useful, but they are not widely measured. However these may also only be useful in liver samples.

Biomarkers need to be as contaminant-specific as possible to be able to establish cause and effect relationships.

The use of biomarkers in stranded animals may also be an issue if the tissues are not in good condition if the animal was unhealthy to begin with, or if they have started to decompose quickly following the stranding. Thus, by-caught and harvested animals may be most useful for the development of biomarker studies.

Metabolite screening in different matrices could be more widely used as biomarkers of contaminant exposure as they are proportional to the body burden, not just indicative of what has entered and gone through the animal. They can therefore give more meaningful information on metabolic processes in the body.

7.0 Suggestions for future work

- 1) Contaminants of concern in cetaceans – The questionnaire will be circulated to experts in the field to identify which contaminants are of highest priority and should be focused on in future modelling efforts.
- 2) Carry out a literature review and database for dose-response relationships of the priority chemicals as identified in 1) above. Which compounds have enough data on dose-response relationships that can be used for future modelling efforts?
- 3) Investigate other testing methods for measuring POPS and other contaminants, e.g. the rapid, inexpensive ELISAs
- 4) Compare the priority contaminant levels in the blubber to other organs to establish the representativeness of live biopsy sampling techniques to obtain tissues that could then be used

assess the total body burden. This has been done for PCBs, but for contaminants that are less lipophilic and for which fat is not the target organ.

- 5) Compare biomarker levels in the blubber to other organs to establish the representativeness of live biopsy sampling techniques to assess the potential impacts of exposure to specific contaminants.
- 6) Investigate the use of different, contaminant specific biomarkers of exposure including metabolites in the faeces.

Finally more investigation into the effects of microplastics in cetaceans is needed including:

- a) The direct (leaching of toxins) and indirect effects (adsorbed contaminants) of microplastic exposure
- b) The feeding strategies of cetaceans may also determine which species may be most at risk enabling areas and priority species to be identified (e.g. Mediterranean fin whale, grey whale, right whale).
- c) In order to assess exposure, we need to quantify ingestion (stomach contents) and excretion (faeces). In terms of surveillance, which strandings schemes are looking for microplastics in the stomachs of stranded cetaceans (both macro and microplastics)?
- d) Using a fresh carcass, investigate the potential biomarkers in the 'target / ideal' organ and then the same biomarker in the skin/blubber. This would help establish the representativeness of blubber biopsies for measuring exposure biomarkers.

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Annex A

Steering Group Members

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