Evaluation of the ecotoxicological effects of POPs and heavy metals, reflecting pathological, microbiological and genetic analyses, on the Mekong River population of Irrawaddy dolphins (*Orcaella brevirostris*)

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I. General Introduction

The Mekong population of Irrawaddy River dolphins (*Orcaella brevirostris*) is one of three riverine populations. All three populations are classified as critically endangered based on IUCN criteria. The current population size is estimated to be 85 individuals in the Mekong River (verbal inf. P. Obrdlik). In addition, since 2003 a total of 88 dolphins were found dead. Therefore, the population of Irrawaddy dolphins is at risk of extinction throughout its range (Dove 2009).

It was documented earlier that adult Mekong Irrawaddy dolphins were by-caught in gill nets. In recent years, an increasing number of immature dolphins were found dead. Different investigations came to the conclusion that the death of young dolphins may be the result of several factors (Dove 2009). These factors include:

1. **Disease**
   a. Aeromonas hydrophila
   b. Other opportunistic bacterial infections

2. **Environmental contaminants**
   a. Organic contaminants
   b. Non organic contaminants such as mercury

3. **Inbreeding depression**
   a. Low genetic diversity

For this report, samples of Irrawaddy River dolphins found dead along the Mekong River between 2006 and 2010 were analysed in different laboratories in Europe and Cambodia (Appendix 1). The aim of the investigation was to gain additional knowledge on histological and toxicological findings as well as on the genetic variation of the Mekong population. The report summarizes those results in relation to previous investigations conducted in Cambodia and the United States of America.
II. Genetics

The genetic investigations were conducted by Dr. Arne Ludwig and Dipl. Biol. Dietmar Lieckfeldt at the Leibniz Institute for Zoo and Wildlife Research (IZW) in Germany. The aim of the study was to analyse the genetic variation of the Mekong population of the Irrawaddy River dolphins. 23 animals were collected between 2004 and 2009. Samples were preserved in ethanol or DMSO, or were frozen.

Two aspects were investigated in the frame of the study: Firstly, the maternal lineage detection based on polymorphisms of the mitochondrial d-loop sequences was performed. Secondly, a screening of nuclear genetic variation based on microsatellites was conducted. All 23 samples yielded PCR fragments suitable for cycle sequencing. Nucleotide differences ranged from one (within *Orcaella*) to 43 (between *Orcinus orca* and *Orcaella heinsohnii*). Phylogenetic calculations revealed a population structure for all three sample sites (Mahakam, Mekong River, coast). Bootstrap support ranged from 58% to 100%. No evidence was found of a female gene flow among the populations. The Mekong River population is dominated by haplotype 1 (96.66%). All other haplotypes are rare. All microsatellites used produced polymorphic band patterns. The dataset is incomplete due to the poor quality DNA in some samples. Failure rate of loci rose with increasing allele sizes. For these reasons, we recommend the development of species-specific, short (<150 bp) microsatellites for further studies addressing pedigree analysis because carcasses found in warm, humid environments carry a substantial risk of low amplification rates.

The results of the new study were also combined with previously available data. It could be shown that four mitochondrial haplotypes occur in the Mekong population, indicating no evidence for a strong decline of maternal lineages. Nevertheless, intraspecific network reconstructions produced evidence of a distinct Mekong River population.
III. Pathology

Results of different pathological investigations were made available for the report. They included necropsy reports as well as histological and microbiological investigations. Unfortunately, only in three cases had all investigations been performed, limiting interpretation of the results.

3.1. Microbiology

Microbiological analyses were conducted at the Institut Pasteur du Cambodge, Laboratoire de biologie medicale, Phnom-Penh. 14 animals were investigated. Sampling locations were reported to be abdomen, abdominal cavity, abscess, amniotic fluid aorta, biopsies, blood, blood vessel, blowhole, blubber lesion, faeces, gangrene muscle lesions, head, hemothorax, kidney, liver, lung, lung abscess, lung granuloma, spleen, under neck, umbilicus, spots on the liver, muscle, neck blubber, neck lesion, neck muscle, thorax, urine, uterus.

The following bacteria were isolated:

- Aeromonas hydrophila
- beta-hemolytic streptococci, group C
- Edwardsiella tarda
- Escherichia coli
- Klebsiella pneumoniae
- Morganella morganii
- Plesiomonas shigelloides
- Proteus vulgaris

All bacteria isolated should be considered potential pathogens. Therefore, it is important to see the occurrence of bacteria in relation to pathological findings.

3.2. Histology

The histological investigations were conducted by Prof. Thijs Kuiken of the Erasmus MC, Universiteit Medisch Centrum Rotterdam, The Netherlands, and Dr. Peter Wohlsein of the Institute for Pathology, University of Veterinary Medicine, Hannover, Germany. A different number of organs per animal were available for histological analyses. Unfortunately, the majority of samples were in a state of advanced decomposition resulting in a loss of cellular structure detail and a strong infiltration of post-mortem bacteria.

In several samples and individuals no abnormalities were detected and no histological diagnoses made. Especially the “gangrenous muscle” of several animals was judged to be post-mortem gas formation with many bacilli present but without oedematous fluid, neutrophils or myocyte degeneration.
Histological findings were:

CID 060912: multifocal stomach ulcerations
CID 061301: focal loss of epidermal tissue in the skin
CID 061302B: focal loss of epidermal tissue in the skin
CID 07009: multifocal skin ulceration
CID 07014: multifocal panniculitis and mild chronic multifocal lymphocytic
CID 09002: acute moderate multifocal suppurative bronchopneumonia
  moderate diffuse hepatic atrophy
  moderate periportal hepatic lipidosis
  acute mild multifocal ulcerative dermatitis
  mild multifocal renal tubular mineralization
  mild anthracosis of the lymph node
CID 09008: acute severe diffuse suppurative leptomeningitis
Sarcocystis species infection of the skeletal muscle
  Moderate focal to diffuse epidermal hyperplasia

The most important findings in relation to the health status of the dolphins were suppurative bronchopneumonia, hepatic atrophy and periportal hepatic lipidosis in one animal and suppurative leptomeningitis in another animal.

The first animal was an adult female. Lesions of the liver can be considered to be primarily of degenerative and secondarily of infectious origin. Large numbers of *Aeromonas hydrophila* were isolated from the lung. These bacteria were also found in several other organs without lesions. As these bacteria are commonly found in the environment of the animals, a postmortem occurrence is conceivable. This is supported by the histological investigations, which indicate that mild autolysis occurred with aggregates of bacteria due to postmortem bacterial invasion. The lesions found, however, were impacting the health status negatively, and the bronchopneumonia should be considered as the potential cause of death.

The second animal was an adult male. Microbiological findings were beta-hemolytic streptococci (group D) in neck muscle, *Citrobacter freundii* in kidney and neck muscle, *Klebsiella pneumoniae* in kidneys, liver, neck and normal muscle, *Escherichia coli* in liver and normal muscle and *Enterobacter aerogenes* in neck muscle and urin in numerous colonies. No histological lesions were associated with the bacteria isolated so that their occurrence need to be considered as irrelevant for the health status. No microbiological investigations were conducted on brain. The acute severe diffuse suppurative leptomeningitis should be considered as a possible cause of death for this individual.

Based on the histological and microbiological findings the cause of death for the other animals remains unclear. A determination of the health status is also impossible because of the poor state of preservation of the histological samples.
IV. Ecotoxicology

4.1. Methodology

4.1.1. Trace elements

Arsenic (As), lead (Pb), cadmium (Cd), iron (Fe), copper (Cu), total mercury (T-Hg), methylmercury (MeHg), selenium (Se) and zinc (Zn) were analysed in the liver of 20 Irrawaddy dolphins (*Orcaella brevirostris*) from the Mekong River. Cd was also analysed in kidney. Galab Laboratories and Institute of Public Health performed all analyses. All data are expressed in fresh weight. Water content of tissues varies in tissues depending on the freshness of the samples (loss of fluid during decay process) and caution should be taken on concentration interpretation. Further analyses should be realized on freeze-dried samples to ensure a certain homogenization of samples.

4.1.2. Organic compounds

The blubber from ten different dolphins were analysed for organic pollutants by Galab Laboratories (Reports A2010104227 and A2010101885). The scope included brominated flame retardants (BFR), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCP), dioxins and furans (PCDD/PCDF), organotin compounds (OTC), perfluorinated tensides (PFOs/PFOA), toxaphene, methyl parathion (Table 1).

Table 4.1. Scope of organic compounds analyzed in the blubber of Irrawaddy dolphins from the Mekong River

<table>
<thead>
<tr>
<th>Organobrominated compounds</th>
<th>Organochlorine pesticides</th>
<th>Dioxin-like compounds</th>
<th>Organic tin compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,3',5,5'-Tetrabromobiphenyl A</td>
<td>1,3-Hexachlorobutadien</td>
<td>1,2,3,4,6,7,8-HpCDF</td>
<td>Di-Decyltin</td>
</tr>
<tr>
<td>Bromocyclen</td>
<td>Aldrin</td>
<td>1,2,3,4,6,7,8-HpCDF</td>
<td>Di-Octyltin</td>
</tr>
<tr>
<td>Decabromdiphenylether, PBDE-209</td>
<td>Chloridan, cis-</td>
<td>1,2,3,4,7,8-HpCDF</td>
<td>Mono-Decyltin</td>
</tr>
<tr>
<td>Heptabrombiphenylether, PBDE-183</td>
<td>Chloridan, trans-</td>
<td>1,2,3,4,7,8-HpCDF</td>
<td>Mono-Octyltin</td>
</tr>
<tr>
<td>Heptabromdiphenylether, PBDE-190</td>
<td>DDD, p,p'-</td>
<td>1,2,3,4,7,8-HpCDF</td>
<td>Tetra-Butyltin</td>
</tr>
<tr>
<td>Hexabrombenzol</td>
<td>DDE, o,p'-</td>
<td>1,2,3,4,7,8-HpCDF</td>
<td>Tri-Butyltin</td>
</tr>
<tr>
<td>Hexabrombiphenyl, PB-153</td>
<td>DDT, p,p'-</td>
<td>1,2,3,4,7,8-HpCDF</td>
<td>Tri-Cyclohexyltin</td>
</tr>
<tr>
<td>Hexabromcisdodecan</td>
<td>Dieldrin</td>
<td>1,2,3,4,7,8,9-HxCDF</td>
<td>Tri-Phenyltin</td>
</tr>
<tr>
<td>Hexabromdiphenylether, PBDE-138</td>
<td>Dienenchlor</td>
<td>1,2,3,4,7,8,9-HxCDF</td>
<td></td>
</tr>
</tbody>
</table>
4.2. Contaminant levels

Irrawaddy dolphins inhabit coastal environments from the Bay of Bengal east to Palawan, Philippines and south to northern Australia (Arnold 2002, Baird & Beasley 2005). They also occur in three large tropical river systems in South-east Asia: the Mahakam (Indonesia), Ayeyarwady (or Irrawaddy, Burma) and the Mekong (Cambodia). Anthropogenic contaminants are ubiquitous in such environment and, being long-lived apex predators, they inevitably accumulate organic and inorganic contaminants across their lifetimes (Kannan et al. 2005). Contaminants are typically considered as a potential contributing factor in marine mammal mortality events, mainly because of their potential impact on immune function, and therefore on population’s susceptibility to disease (Siebert et al. 1999, Ross 2002, Jepson et al. 2005, Hall et al. 2006).

Inorganic contaminants include metals, metalloids, and a number of small molecules such as phosphate and ammonia (Wright and Welbourn 2002). In contrast to organic contaminants, many trace elements occur naturally as a consequence of naturally occurring processes (e.g. geological weathering, degassing of the earth’s crust and oceans, volcanic activity) and as a result of anthropogenic activities (Pelletier 1985, Zyadah & Abdel-Baky 2000, Wright & Welbourn 2002, DeForest et al. 2007). Another point of contrast between organic and organic contaminants is that some elements such as Zn, Cu and Fe are required as nutrients and display a key role in organisms. They are regulated through homeostatic processes. However at higher doses, essential element excess begin to have harmful effects (Wright and Welbourn 2002). Cd, As, Pb and Hg have no known function in organisms and are therefore considered as toxic even at low concentrations. Hg concentrations can be elevated in livers and kidney of marine mammals and is generally associated to Se as a consequence of a complex demethylation process occurring in liver (Nigro & Leonzio 1996, Ikemoto et al. 2004).

Essential elements such as Fe, Cu, and Zn analysed in the liver of Irrawaddy dolphins (Table 4.2.) are from the same order of magnitude as compared to other marine mammal species (Bennet et al. 2001, Das et al. 2003).
Table 4.2. Trace element concentrations in the tissues of *Orcaella brevirostris*.

Data are expressed as mean (median) ± standard deviation, min-max and n (number of analysed samples). All concentrations are expressed in mg.kg⁻¹ fresh weight.

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>As</th>
<th>Pb</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.6 (0.1)±6.6</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.002-19</td>
<td>&lt;0.002-19</td>
<td>&lt;0.002-19</td>
<td>&lt;0.002-19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n= 13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td><strong>0.29</strong> (0.04)±0.38</td>
<td><strong>0.08</strong> (0.06)±0.07</td>
<td><strong>0.04</strong> (0.02)±0.06</td>
<td><strong>578</strong>[458] ±551</td>
<td><strong>25</strong>[7]±31</td>
<td><strong>51</strong>[44]±29</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0025-1.0</td>
<td>&lt;0.025-0.24</td>
<td>&lt;0.007-0.21</td>
<td>60-2369</td>
<td>1.7-111</td>
<td>19-110</td>
</tr>
<tr>
<td>n= 13</td>
<td></td>
<td>n= 11</td>
<td>n= 12</td>
<td>n= 19</td>
<td>n= 18</td>
<td>n= 19</td>
</tr>
</tbody>
</table>

Except for one individual (OBRE 06-09-12), zinc concentrations detected in the liver of dolphins from the Mekong River are within the normal range for marine mammals (Law et al. 1991; Das et al. 2004). Zinc is an essential element, and consequently animals will maintain the concentration within a specific range by homeostasis. Part of the observed variability of the results can be linked to the water content of the tissues (which may vary widely regarding the freshness of the carcass during the sampling procedure). Law et al. (1991) suggested a homeostatic range of 20-100 µg.g⁻¹ wet weight for zinc in liver tissue in common porpoise (*Phocoena phocoena*), and postulated that animals outside of this range are those whose regulating mechanism may be impaired (Law et al. 1991). Other essential elements are within normal range. Cd, As and Pb are low in the tissues, except for three dolphins displaying elevated level of Cd in the kidney (9, 14 and 19 mg.kg⁻¹). Cd concentrations in the kidney of other dolphins remained lower (below 3 mg.kg⁻¹). Cd is known to accumulated with age and therefore, these three adult females might be older females.

High Hg concentration have been documented in the liver and kidney of marine mammals (from both pristine and contaminated areas), generally associated to Se in a non organic form (tiemannite or HgSe) (Nigro & Leonzio 1996, Woshner et al. 2002, Das et al. 2003, Ikemoto et al. 2004, AMAP 2005). The source of mercury in the Mekong river is thought to be from gold mining activities along the Mekong river (Dove 2009).

T-Hg concentration in the livers of Irrawaddy dolphins from the Mekong River ranged from 0.4 to 18 µg.g⁻¹ fresh weight (Table 4.3). Higher concentrations of Hg ranging from 1 to 344 µg.g⁻¹ dry weight (around 0.25 to 85 µg.g⁻¹ fresh weight assuming a mean water content of 75%) were previously described in the liver of stranded harbour porpoises from the southern North Sea (Das et al. 2004). The high relevance of tiemannite precipitation in marine mammals (compared to other mammals and birds) is likely to be related to a combination of factors, namely, elevated MeHg exposure, due to fish eating habits, and the inability to excrete MeHg through gills, feathers or fur (Nigro et al. 2002, Woshner et al. 2002, Ikemoto et al. 2004, Sonne et al. 2007). This complex process results generally in a 1:1 Se:MeHg molar
ratio combined to a low percentage of methylmercury in adult marine mammals (Wolfe et al. 1998, Siebert et al. 1999) though T-Hg concentration can reach high concentrations. Here, the ratio of selenium to total mercury (Se:Hg) and Se to inorganic-mercury ratio (Se:I-Hg) shows that Se is in excess compared to mercury and also that a only a fraction of selenium is bound to inorganic mercury. The fact that the percentage of MeHg is elevated (from 17 to 97% in adults) is unusual and raises questions about potential toxicity.

Table 4.3. Total mercury (T-Hg), methylmercury (MeHg) and Se concentrations in the liver of Orcaella brevirostris. % of MeHg was also calculated as well as the selenium to Hg molar ratio

<table>
<thead>
<tr>
<th></th>
<th>T-Hg</th>
<th>Me-Hg</th>
<th>%MeHg</th>
<th>Se</th>
<th>Se:T-Hg</th>
<th>Se:I-Hg*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>4.4 ± 5.6</td>
<td>2.6 ± 4.3</td>
<td>52 ± 30</td>
<td>2.1 ± 1.4</td>
<td>1.9 ± 1.2</td>
<td>7 ± 11</td>
</tr>
<tr>
<td></td>
<td>0.4-18</td>
<td>0.1-15</td>
<td>16-97</td>
<td>0.5-6.9</td>
<td>0.6-5.8</td>
<td>1-48</td>
</tr>
<tr>
<td></td>
<td>n=19</td>
<td>n=18</td>
<td>n=18</td>
<td>n=19</td>
<td>n=19</td>
<td>n=18</td>
</tr>
</tbody>
</table>

Regarding organic contaminants, DDTs were the predominant compounds found in Irrawaddy dolphin tissues. A DDT concentration greater than 180,000 ng.g⁻¹ lipid weight was found in the blubber of one individual (OBRE05-06-01). DDT concentrations ranged from 10 to 18,0086 ng.g⁻¹ (or µg.kg⁻¹) lipid weight. The lowest concentration was detected in a new born. These levels are higher than DDT concentrations reported in the blubber from Irrawaddy dolphins from Bay of Bengal in India where DDT concentration ranged from 310 to 10,000 ng.g⁻¹ lipid weight (Kannan et al. 2005). These high levels reflect DDT use for malaria control in Asia. Among DDTs, p,p’-DDE was the predominant compound in the tissues of Irrawaddy dolphins from the Mekong river as it was also for dolphins from India (Kannan et al. 2005). A high proportion of p,p’-DDE in biota would suggest exposure to aged residues from the environment and greater metabolic transformation by the organism (Kannan et al. 2005). Other pesticides namely chlordane (cis and trans), nonachlor, hexachlorobenzene, oxychlordan, metoxychlor, mirex, camphechlor, aldrin, dieldrin, diconchlor, endosulfan (alpha and beta) endosulfansulfat, endrin, HCH (alpha, beta, delta, epsilon) and octachlorstyrol, were also analysed in selected individuals. Most of the concentrations were below the detection limit except for trans nonachlor > hexachlorbenzol followed by metoxychlor> oxychlordan >mirex. Toxicity levels for these compounds have not been assessed for marine mammals but the fact that they are detectable in tissues if Irrawaddy dolphins reflect their proximity to pollution. One should paid attention to
Camphechlor, which was surprisingly high in one dolphin (CID 09 001, 163 µg.kg⁻¹). Camphechlor (or toxaphen), a non-systemic insecticide, displays a complex composition with at least 202 different congeners identified. Camphechlor is a reaction mixture of chlorinated camphenes containing 67-69% chlorine. However, the meaning of such a value in term of toxicity is rather difficult to assess due to poor sampling size.

Dioxins and furans were analysed in blubber and of the most abundant congener determined was OctaCDD, with values ranging from 1.5 to 56 pg.g⁻¹ lipid weight. The results of this study indicate low values (TEQ and concentrations) with an average of only 5 out of 17 congeners detected. Generally, the TEQ concentrations for the Mekong dolphins were orders of magnitude lower than levels previously described for marine mammals from Northern hemisphere (e.g. (Das et al. 2008a) and for Ganges River dolphins from India in which TEQs exceeded the threshold value of 160 pg TEQ/g assessed for mink (Senthilkumar et al. 1999).

PCBs were the second most abundant (after DDT compounds) while other pesticide levels were relatively low though always detectable. Concentrations of PCBs in Irrawaddy dolphins ranged from 85 to 950 µg.kg⁻¹ (ng.g⁻¹). PCBs were found at concentrations ranging from 28 to 390 ng.g⁻¹ lipid weight in blubber from Irrawaddy dolphins sampled in Bay of Bengal, India (Kannan et al. 2005). A threshold PCB concentration of 8700 ng.g⁻¹ lipid weight has been reported to generate physiologic effects in aquatic mammals (Kannan et al. 2000). More recently, a threshold of 17 µg.g⁻¹ (or 17 000 ng.g⁻¹ lipid weight) has provided valuable benchmark for interpreting whether associations between disease and PCB exposure will be biologically significant (Jepson et al. 2005). Concentrations of PCBs in Irrawaddy dolphins were one to two orders of magnitude lower than this threshold concentration.

Among all brominated compound analysed, BDE 47 (a polybrominated diphenyl ether), was the most abundant congener in analysed samples except for one individual (CID 09 005, an adult female) where BDE 100 and BDE 99 were found at high concentrations (802.7 and 178.7 ng.g⁻¹ lipid weight respectively compared to 123.5 ng.g⁻¹ lipid weight for BDE 47). BDE 47, the most persistent congener in marine mammals ranged from 14 to 938 ng.g⁻¹ lipid weight (or 0.014 to 0.938 µg.g⁻¹ lipid weight) The concentrations of BDE 47 in the Irrawaddy dolphins are one to three orders of magnitude lower than concentrations reported for harbour porpoises from the North Sea (0.11-3.88 µg.g⁻¹ lipid weight)(Weijs et al. 2009) but similar to that observed in blubber of Irrawaddy dolphins from India (from 0.98 to 18 ng.g⁻¹ lipid weight) (Kannan et al. 2005). The low levels of both PCBs and PBDEs could be explained by the rural nature of Cambodia but the detection in Irrawaddy dolphins confirms their widespread distribution all over the world. However, lipophilic substances such as
DDT, PCBs and PBDEs rely also on body condition and nutritional status (Debier et al. 2006) and comparison of concentrations between studies should be realized with caution.

In recent years, tributyltin (TBT) and its degradation products, monobutyltin (MBT) and dibutyltin (DBT) have received attention due to high toxicity of TBT (Tanabe 1999). Total butyltin concentrations detected in livers of marine mammals ranged typically from 1 to 10 µg.g⁻¹ wet weight (from 0.001 to 0.01 µg.kg⁻¹). Concentrations in blubber are generally lower due to high affinity of tin compounds to hepatic proteins. Concentrations in Irrawady dolphins from the Mekong river are higher (from 50 to 120 µg.kg⁻¹ or ng.g⁻¹) than these typical described values. Butyltin concentrations ranging from 360 to 1800 ng.g⁻¹ wet weight were described in the blubber of Ganges River dolphins (Kannan et al. 1997). Accumulation of butyltin residues in Irrawaddy dolphins suggests the presence of sources and inputs in the Mekong River. TBT is not only associated to antifouling paints (boat activities) but also in paint manufacturing factories and as slimicide and biocide in paper manufacture. The principal commercial use of butyltin is in the stabilization of TBT (Kannan et al. 1997). Range of concentrations detected in Irrawaddy dolphins reflects current use of TBT and raises questions about toxicity as these compounds may affect endocrine and immune systems of vertebrates, even at low concentration (Tanabe 1999).

Perfluorinated compounds (PFCs) including perfluorinated acids are today widely distributed in the marine environment (Yamashita et al. 2005) and were described in many species of marine mammals including river dolphins (Van de Vijver et al. 2003, Van de Vijver et al. 2004, Van de Vijver et al. 2005, Van de Vijver et al. 2007, Dorneles et al. 2008, Yeung et al. 2009, Butt et al. 2010). They have broad application spectrum as surfactants, adhesive, fire retardants, agrochemicals and food packaging. Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are the two typical PFCs found in the environment (Yamashita et al. 2005, Yeung et al. 2009). PFOA and PFOS were below the detection limit in the blubber for all analysed Irrawaddy dolphins. However these compounds do not typically accumulate in blubber of marine mammals. Unlike most persistent organic pollutants, and due to their surfactant like structure, PFCs accumulated to a higher extent in kidney and liver compared to blubber (Van de Vijver et al. 2005). PFOS was the predominant compound analysed in the liver of Ganges River dolphins with an arithmetic mean of 27 ng.g⁻¹ wet weight (Yeung et al. 2009). PFCs concentrations in biota collected in waterbodies from India were lower than values reported for China, Brazil, USA and Europe Western countries (Van de Vijver et al. 2003, Dorneles et al. 2008, Yeung et al. 2009). Low PFCs levels analysed in Irrawaddy dolphins from the Mekong River reflect therefore the low PFC pollution of the Mekong River. However, long-term monitoring of Irrawaddy dolphins and other aquatic species should be seriously envisaged in relation to industrialization and economic development.
V. Conclusions and recommendations

5.1. Genetics
It can be concluded that the Mekong population harbors a sufficient level of genetic variation, which currently makes inbreeding a lesser risk for their extinction. However, conservation programs should lead to the preservation of the entire genetic variation of the Mekong population. The transfer of animals from the coastal population and/or the Mahakam population should be avoided because our data indicates a genetic separation of the Mekong dolphins from both the coastal population and the Mahakam dolphins. A release of dolphins from these populations would risk outbreeding depressions in Mekong dolphins. In general, populations evolved allelic patterns adapted to their local environment. The release of non-native specimens often results in the loss of locally adapted allelic complexes causing reduction in fitness of individuals.

5.2. Pathology
The results of the histology and microbiology do not support the hypothesis that Irrawaddy dolphins from the Mekong River suffer from Aeromonas hydrophila or other bacterial infections. It cannot be excluded that more lesions could have been found with samples in a better state of preservation. The gangrenous neck lesions were judged to be postmortem lesions caused by autolysis. Main infectious pathological findings were suppurative bronchopneumonia in one animal and suppurative leptomeningitis in another. It was not possible to detect effects of immunosuppression on the investigated material and with the methods used.

5.3. Chemical pollution
Chemical pollution, in addition to habitat destruction, is a significant factor in the decreases in river dolphin populations (Reeves 2002). Irrawaddy dolphins may be vulnerable to toxic effects due to their close proximity to pollution sources and their lesser capacity to metabolize contaminants (Kannan et al. 2005). Due to numerous confounding factors, it is not possible to relate levels of pollutants to observed pathological lesions. However, it is likely that chemical contaminants do adversely impact on the health of the Irrawaddy dolphins at present, and have also affected previous generations. We note that the adverse effect of contaminants may be insidious, and could potentially combine with other stressors to have an overall effect that influences the incidence and severity of infectious disease. In general, the residue levels of organochlorines and PBDEs in Irrawaddy dolphins from the Mekong River are lower than the concentrations reported for other cetaceans in the coastal and riverine waters of Asia, except for DDT, which remain high reflecting its use against malaria in Asia. The high percentage of organic mercury is unusual and we cannot provide any explanation for such a result. Methylmercury is immunotoxic, even at low concentration
(Das et al. 2008b). Because the habitat of Irrawaddy dolphins is vulnerable to degradation, further efforts to conserve this endangered species should focus on establishing protected areas, raising public awareness, managing fisheries to decrease incidental killings and ensure the sustainability of prey species, as well as decreasing chemical pollution. As the number of animals is already extremely low, no time is left to establish appropriate tests for this species and to wait for the opportunity of sampling animals alive or shortly after death. The consequences of high pollutant levels are already firmly established through the results of research on other marine mammals and terrestrial species.

Efforts should be directed at immediate improvement of the animals’ natural environment. Artificial insemination is not needed as the population harbors a sufficient level of genetic variation. If the environmental improvements cannot be realized in the short term, other programs e.g. taking animals to an artificial environment should be established to preserve the last Irrawaddy dolphins from the Mekong River.
References

AMAP (2005) Heavy Metals in the Arctic. In: AMAP (Arctic Monitoring and Assessment Programme) Assessment 2002 Oslo, Norway


Appendix 1: List of the available and evaluated original analytical results and reports

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<th>Category</th>
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<td>Trace elements</td>
<td>Galab Laboratories Geesthacht, Germany; Institute of Public Health Ostrava, Czech Republic</td>
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<td>Persistant Organic Pollutants</td>
<td>Galab Laboratories, Geesthacht, Germany</td>
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<td>Pathological investigation</td>
<td>Universiteit Medisch Centrum Rotterdam, The Netherland – Prof. Thijs Kuiken; Institute for Pathology, University of Veterinary Medicine Hannover, Germany – Dr. Peter Wohlsein</td>
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<td>Microbiological analyses</td>
<td>Institut Pasteur du Cambodge, Laboratoire de biologie medicale Phnom Penh, Cambodia</td>
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<td>Necropsy reports</td>
<td>Cambodian Mekong Dolphin Conservation Project Kratie, Cambodia – Dr. Verne Dove and Dr. Martin Gilberts</td>
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