Development of otter-based quality objectives for PCBs (DOQOP): Overview of the project in view of the environmental policy aims

Martine E.J. van der Weiden[1], A. Jan Hendriks[2], Mariette H.J. Klein[3], Pim E.G. Leonards[4], Maarten Smit[5], Tinka A.J. Murk[6] Erik van de Plassche[7], Trudy Crommentuijn[7], Robert Luttik[7] and Bert van Hattum[4]

1 Ministry of Housing, Spatial Planning and the Environment, Risk Assessment and Environmental Quality Division, P.O. Box 30945, 2500 GX The Hague, The Netherlands. Email:m.vanderweiden@dsvs.dgm.minvrom.nl
2 Ministry of Transport and Public Works, Institute for Inland Water Management and Waste Water Treatment; P.O.Box 17; 8200 AA Lelystad, The Netherlands.
3 Ministry of Agriculture, Nature Management and Fisheries, National Reference Centre for Nature Management; P.O. Box 30; 6700 AA Wageningen, The Netherlands.
4 Institute for Environmental Studies, Vrije Universiteit, De Boelelaan 1115, 1081 HV Amsterdam
5 Dutch Otterstation Foundation, Groene Ster 2, 8926 XE Leeuwarden, The Netherlands.
6 Department of Toxicology, Agricultural University, Wageningen, The Netherlands
7 National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

1. Abstract
In this paper an overview is given of the project 'Development of Otter(*Lutra lutra*)-based Quality Objectives of PCB's (DOQOP)' from a policy point of view. The aims of this project are explained in the light of the environmental policy targets from the three supporting ministries: Ministry of Housing, Spatial Planning and the Environment, the Ministry of Transport and Public Works and the Ministry of Agriculture, Nature and Fisheries.
The aim of the DOQOP project is to examine if the otter is sufficiently protected by the current method of criteria setting, taking into account secundary poisoning. The second aim is to find out if the PCB concentrations in former Dutch otter habitats are low enough to achieve a succesful reintroduction of captive-bred otters in the Netherlands.
The DOQOP project consists of two parts: the first part consisted of the compilation of two reports with the available data about otters and mink. The aim of the first report was to find out if there are enough data available to derive a well-funded No Observed Effect Level (NOEL) for PCBs in sediment, water or food for the otter. In the second report a critical evaluation was made if there are sufficient data available on experimental studies to calculate critical levels in the mink (*Mustela vison*), a species closely related to the otter.
Secondly, in continuation on these reports and because of the lack of necessary data for otters a field study is started. In this field study PCB contents in otters from high, intermediate and low PCB-exposed areas are analyzed including their environment (sediment and prey). In addition physiological parameters in otter blood plasma and liver are measured. Preliminary results of this field study are presented.

2. Environmental policy in The Netherlands

Dutch system of criteria setting: Procedure for direct effects

The Dutch government aims with their environmental policy to achieve and remain a sustainable environment. The strategy to reach this aim is described in the National Environmental Policy Plan 2[1], and includes e.g. the derivation of effect-based criteria for the ecosystem, assessment of the environmental quality and achievement of the desired environmental quality by measures such as emission reductions for several compounds.

In order to assess the possible effects of certain compounds on a ecosystem a procedure to derive effect-based criteria has been developed by the National Institute of Public Health and the Environment[2]. First, suitable available ecotoxicological data (NOECs) are collected, involving parameters which are of importance for the survival of the species in the ecosystem, such as growth, reproduction and lethality. If there are more than 4 chronic or long term data for different taxonomic groups available a Maximum Permissible Concentration (MPC) is calculated, using a statistical extrapolation[3]. If less than 4 data are available the modified EPA method is applied to calculate a MPC, using fixed security factors[4].

After derivation of the MPCs for a certain compartment (soil, water, sediment and air) these MPCs are intercompartimentally harmonized, in order to avoid exceedance of the MPC from one compartment by the MPC from another compartment through physico-chemical exchange processes, using the Simplebox model[5]. With these final intercompartimentally-harmonized MPC's the Dutch Government aims to define a concentration per compound below which no adverse effects will occur for organisms in the environment. In order to take combination toxicity into account a Negligible Concentration (NC) has been defined, which is the MPC divided by 100. Below this NC the risks for the ecosystem caused by chemicals are considered negligible. Currently the National Institute of Public Health and the Environment is working at the derivation of MPCs and NCs for approximately 200 compounds, including PAHs, pesticides, volatile compounds, metals and PCBs. Based on these MPCs and NCs and after taking several other aspects into account, such as economical and technical aspects, detection limits, current environmental quality and natural background concentrations of metals, limit and target values are set. These MPCs and NCs and limit and target values are used to assess environmental quality and to judge if the emission reduction measures are sufficient to achieve and remain a sustainable environment.

Dutch system of criteria setting: Procedure taking into account secundary poisoning

For the relevant compounds with a MW between 500 and 700 and a log Kow>4 and some metals a special correction is made in the procedure of criteria setting. These persistent and toxic compounds show accumulation in biota and transport through the food chain, a proces known as secundary poisoning. For instance PCB's are persistent and toxic pollutants, which are widespread in the environment. Due to their lipophilic and persistent properties a background contamination of PCB's has been demonstrated everywhere in biota, e.g. plankton, mollusc, crustaceans and fish. These compounds accumulate through the foodchain and high levels can be reached in organisms at the top of the food chain, top predators, such as larger marine and freshwater fish species and fish-eating birds[6]. With the method to calculate a MPC, taking into account secundary poisoning, the calculated MPCs aim to protect all organisms including top predators[7,8]. With this method an aquatic route has been defined: water→ fish or mussel→ fish- or mussel-eating bird or mammal. The MPC is then calculated as follows: $MPCwater = \text{extrapolated No Effect Concentration}_{bird\ or\ mammal}/BCF_{fish}$. The BCF_{fish} is defined as the concentration of a compound in fish divided by the concentration in the water. In addition, a correction factor is used for differences in caloric contents of food between organisms in the laboratory and the field. To find out if secundary poisoning is critical for that compound these MPCs taking into account secundary poisoning for higher organisms are compared with the MPCs for direct effects derived via the normal procedure, without taking into account secundary poisoning. The lowest MPC is chosen.

The aim of the DOQOP project is to contribute underlying data for the derivation of the MPCs and NCs for PCBs. With the results of DOQOP a No Observed Effect Concentration (NOEC) for the otter can be calculated and corresponding No Observed Effect Levels (NOELs) for its environment (sediment, water and prey). Furthermore with the results from this DOQOP project an evaluation can be carried out if the method for derivation of MPCs and NCs for PCBs, taking secundary poisoning into account, gives sufficient protection to a probably very sensitive top predator such as the otter.

Nature conservation Policy in the Netherlands
In reaction to the growing concern about the deterioration of the natural environment, the Dutch government has developed the Nature Policy Plan in 1990[9]. In this plan the main objective is 'a sustainable conservation, rehabilitation and development of nature and landscape'. The Nature Policy plan focuses on the establishment of a 'National Ecological Network', a network of existing and still to be developed nature areas, and maintaining and improving the biodiversity. Species that are of national and international interest are target species for the Nature Policy Plan. Within this aim the Dutch policy has adopted a plan aiming at the return of the otter in the Netherlands. In recent years only rarely some otter tracks have been found and the species is considered to be nearly extinct. The restoration plan for the otter includes the preparation of former otter habitats in terms of improvement of water and sediment quality, removal of disturbances, creation of migration corridors between several areas and potential reintroduction of captive-bred otters[10]. Before this can take place information is needed about the environmental quality, which is necessary for the future Dutch otter population to survive and for the otter population to thrive. From literature it is known that PCBs may play an important role in the decline of the otter in large parts of Europe [10,11]. Partly based on this aim the DOQOP project was started in 1993.

3. The DOQOP project: Literature research
First of all two reports (reviews) were prepared on the available knowledge (state of the art). The first report was a compilation of the available data on the contamination of PCB's in the environment in relation to PCB levels and effects in the European otter(populations)[10]. The aim of this report was to find out if there are enough data available to derive a well-funded NOEL for PCBs in sediment, water or food for the otter.
From this review it is concluded that the data from different authors from different countries are difficult to compare, because of the variations in organs for analysis, the format of the data, the analysis, the type of PCBs which are analyzed etc. In addition, there are no data available about the bioaccumulation of sediment-bound PCBs in prey organisms and transport of PCBs through the food chain resulting in accumulation in the otter. Furthermore there is no knowledge about a dose-response relationship between the internal PCB concentration in otter organs and physiological sublethal effects, and effects on population status[10].

In the second report a critical evaluation was made of the available data of experimental studies of the mink (*Mustela vison*), a species closely related to the otter[12,13].
There are sufficient data available to calculate EC_{50}s and EC_1s for reproductive effects of PCBs in the mink as shown in table 1.A level of 50 μg PCBs/g lipid is generally assumed as a critical level for the mink. Recently a study has been published confirming the high sensitivity of mink to the reproductive toxicity of dioxin-like compounds[14]. The critical levels for mink are often generally assumed to be applicable for the otter as well. However, although the mink and the otter are closely related as mustelid species, there are not enough data to prove if they actually possess a comparable sensitivity towards PCBs.

	Mink Tissue Litter Size	Kit survival	Mink Diet Litter Size	Kit Survival
Critical level(EC_{50})				
total PCB $\mu g/g$ (ww)	1.2	2.36	0.371	0.730
PCB 153 $\mu g/g$ (ww)	0.16	0.22	0.051	0.068
TEQ_{tot} pg/g (ww)	160	200	77	96
NOEC (EC_1)				
total PCB $\mu g/g$ (ww)	0.47	1.29	0.145	0.399
PCB 153 $\mu g/g$ (ww)	0.084	0.16	0.026	0.049
TEQ_{tot} pg/g (ww)	105	35	50	17

Table 1: Proposed critical concentrations (EC_{50}) and NOECs (EC_1) of total PCB, PCB 153 and TEQ (according to Safe TEFs) for the mink and according corresponding NOELs in the mink diet for reproductive effects[13].

4. The DOQOP project: Field study

Aims of the study

Based on the above described lack in knowledge the DOQOP project was continued with a field study involving PCB analyses of sediment, prey and otter samples and measurement of physiological effects in the otter and thus collect the necessary data.

The (sub)aims of this second part of the DOQOP project are listed below:

1 Get information about the dose-response chain: PCBs in sediment→ PCBs in prey→ PCBs in otter→ physiological effect in the otter

2 Calculation of a NOEC for the otter and NOELs in water, sediment and prey for PCBs.

3 Based on the calculated NOEC and NOELs for PCBs a prediction is made if the otter can be reintroduced in its former habitats in the Netherlands. These areas are the 'Oude Venen' and the 'Oostervaarderplassen'.

4 Contribution of underlying data for the derivation of the MPCs and NCs for PCBs, such as biomagnification factor (BMF) from prey to otter, Bioconcentration Factor Sediment Fish (BCSF) from sediment to prey, dose-effect curve for PCBs and physiological effects in the otter. Furthermore with the results of this DOQOP project an evaluation can be carried out if the method for derivation of MPCs and NCs, taking into account secundary poisoning, gives sufficient protection to a very sensitive top predator such as the otter.

5 Find a possible correlation between the status of the otter population and the PCB content in the otter and its environment.

6 Make a comparison between the effects of PCBs between the otter and the mink in order to fund the assumption that sensitivity to PCB toxicity of these closely related species is comparable.

7 Development of feasibility of exposure parameters for monitoring of effects in otters caused by PCBs

8 The representativity of the 7 standard PCBs as marker for total PCB content or TEQs will be evaluated.

Experimental design

Otters are collected from Danmark (low PCB-exposed group), Sweden (high- and intermediate PCB-exposed group) and Austria (high PCB- exposed group). From the sampling area in Danmark samples of sediment and prey (several fish samples) were collected. PCB analyses are now in progress in order to get toxicokinetic data about secundary poisoning in the otter, such as congener-specific BCSFs and BMFs. The PCB contents in sediment, liver and blood of otter and fish have been analyzed by the Calux Luc-TEQ method[15] and GC-ECD and GC-MS[16].

Furthermore physiological parameters were measured in the liver and blood plasma of the otter, such as the Vit A content (retinol/retinylpalmitate) and its transport carrier TT4 in the blood plasma. In previous studies it has been shown that these parameters play an important role in physiological processes, such as metabolisme, resistance against diseases, growth, reproduction etc. Furthermore it has been shown that these parameters are specificly effected by PCB metabolites[17].
As far as the information is available the PCB content and physiological effects of the animals will be correlated to possible pathological status of the organisms and the status of the otter population.

Preliminary results and discussion
The field study of the DOQOP project is still in progress, therefore not for all these above defined aims results can be presented yet. In addition, these results are presented more in detail by van Hattum (overview field study), Leonards (PCB analysis, toxic risks of planar PCBs in otters) and Murk (Calux assay, physiological parameters) at the Dioxin 1996 conference.
The headlines of the results until now will be listed below in the sequence of the (sub)aims at the previous page.
PCB's were analyzed in the otter, fish and sediment samples in Danmark. The correlation between the analyses from the Calux assay and the GC-TEQs are highly significant. With these results toxicokinetic data about the transport in the food chain, such as the BMF from fish to otter liver and BCSF from sediment to fish can be calculated for 14 PCB congeners. The BCSF for the sum of 7 standard PCBs (28,52,101,118,138,153,180) is approximately 13. An interesting and striking result is that the BMF from fish to otter for the toxic planar PCB 126 and PCB 169 are very high (approximately 110 and 150, respectively), indicating that the otter does not metabolize these compounds, even more that these compounds get stored in the liver.
With a part of the data analyzed it is already obvious that a significant negative correlation is found between the TEQ contents and the Vit A content (retinol/retinylpalmitate) in liver and blood plasma of the otter. This correlation is much better when expressed at TEQ than when expressed as sum of PCBs, indicating the influence by non- and mono-ortho PCB's on the decrease in Vit A content. When all the samples are analyzed it is probably possible to determine a NOEC for PCB's for this effect for the otter, and calculate this into corresponding NOELs for prey or sediment.
With these preliminary results it is shown that the decrease in retinol content in the liver of the otter is more severe than in the mink.
As exposure parameters to monitor these effects of PCBs in otters, the measurement of retinol/retinylpalmitate contents in the liver appears to be better suitable and less variable, than the retinol content in the blood plasma.
These data of toxicokinetics in the food chain (BCSFs, BMFs) and the dose-response of the internal PCB contents with physiological effects (resulting in a NOEC for the otter) are very useful in the derivation of a MPC and NC for PCBs, taking secundary poisoning into account. Furthermore with the calculated NOECs for the otter and NOELs for the prey and environment, using the results of this field study, it can be validated if the MPC and NC for PCBs for direct effects and the MPC and NC, including secundary poisoning, give enough protection to a sensitive toppredator such as the otter.
The PCB contents in the former otterhabitats in the Netherlands are in most cases at the same level and in some cases higher than the sediment concentrations in the habitat of the thriving Danish otterpopulation (results not presented here). More analyses are needed to give a conclusion whether or not the PCB contents in the Dutch former otter habitats will harm the succeedance of the reintroduction of the otter in the Netherlands.
For the remaining aims of the DOQOP project it is at this stage of the study too early to draw conclusions and more samples have to be analyzed.

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