Effect on retinoid homeostasis in the flounder (*Platichthys flesus*) following chronic exposure to contaminated harbour dredge spoil from Rotterdam harbour.

H.T. Besselink, A. Brouwer.
Department of Toxicology, Wageningen Agricultural University,
Tuinlaan 5, 6703 HE Wageningen, The Netherlands
A.D. Vethaak.
Ministry of Transport, Public Works and Water Management, Directorate-General for Public Works and Water Management, National Institute for Coastal and Marine Management/RIKZ, Ecotoxicology Section. P.O. Box 8039, 4330 EA Middelburg, The Netherlands

1. Abstract
Flounder (*Platichthys flesus*) were chronically exposed to either a mesocosm containing relatively clean sand from the Dutch Wadden Sea, a mesocosm loaded with contaminated dredge spoil from Rotterdam harbour, or a mesocosm loaded with relatively clean sand from the Dutch Wadden Sea and a shared water circulation with the contaminated mesocosm. After three years flounder were analysed for plasma and hepatic retinoid levels. Liver retinyl palmitate stores were affected only in the indirect polluted mesocosm. In contrast a significant reduction in plasma and hepatic retinol levels was observed in both the direct and indirect contaminated mesocosms. The present results clearly demonstrate that retinoid levels are affected by long-term exposure of flounder to environmental pollutants.

2. Introduction
Flounder (*Platichthys flesus*) inhabiting Dutch coastal waters and estuaries show relatively high incidences of skin disorders and liver tumours[1,2]. A number of authors have attributed such adverse health effects to high concentrations of environmental pollutants, e.g. polycyclic aromatic hydrocarbons (PAHs) and polyhalogenated aromatic hydrocarbons (PHAHs)[2,3]. From mammalian studies it is known that PHAHs suppress the immune system and induce dermatological alterations[4,5]. In addition PHAHs are well known tumour promotors[6].

A possible involvement of the retinoid metabolism in the toxicity of PHAHs in mammals has been suggested because of the resemblance between symptoms associated with hypovitaminose A and the toxicopathological effects of PHAHs[7]. In addition, it has been shown that retinoids suppress the process of carcinogenesis and to modulate PHAH induced carcinogenesis[8,9]. A number of studies showed that PHAHs can alter retinoid levels in mammals[7,10]. No dose-related changes in retinoid levels were found in flounder upon short-term exposure to either TCDD or Clophen A50, a commercial mixture of PCBs (Besselink *et al.* submitted for publication)[11].

To investigate the long-term effects of environmental pollution on plasma and hepatic retinoid levels, flounder were kept in three large mesocosms with different pollution levels for 36 months. The results of this study are presented in this paper.

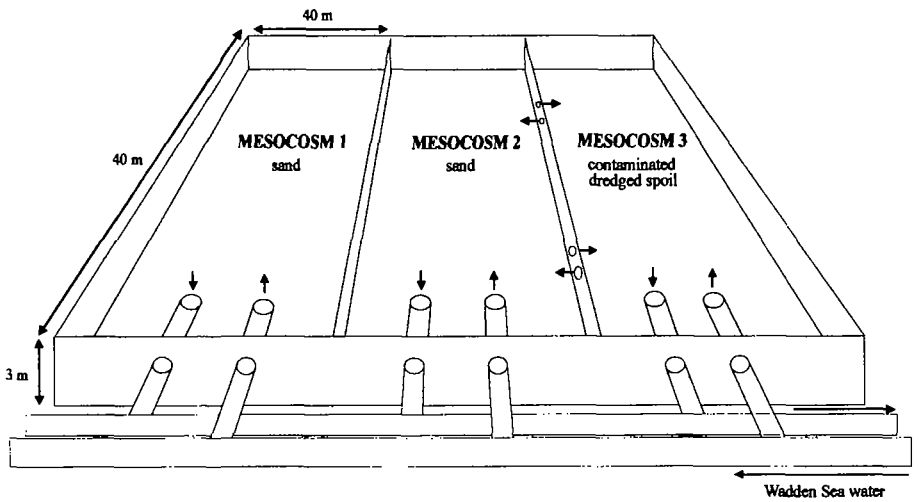

Fig. 1.
Schematic view of the three mesocosms. Mesocosm 1 contained relatively clear Wadden Sea sand, with water abstracted from the Dutch Wadden Sea. Mesocosm 2 also contained relatively clean Wadden Sea sand, but the water shared a common circulation with mesocosm 3. Mesocosm 3 was loaded with contaminated dredged spoil from Rotterdam harbour. The water was taken from the Dutch Wadden Sea. Drawing according to Vethaak *et al.*[1].

3. Methods

Experimental design. Three large-scale mesocosms were loaded as follows (Fig. 1). Mesocosm 1: relatively clean sandy sediment and water from the Dutch Wadden Sea. Mesocosm 2: the same sediment as mesocosm 1 but sharing a common water circulation with mesocosm 3. Mesocosm 3: contaminated dredged spoil from Rotterdam harbour. The mesocosms were stocked with either 1200, 1200, or 400 1-year-old flounder captured in the Dutch Wadden Sea[1]. After three years of exposure, flounder were collected, anaesthetised and blood was collected as described by Janssen *et al.*[12]. Blood was centrifuged at 800 g in a Eppendorf centrifuge and the plasma collected and stored at -20°C until analysis. After decapitation of flounder, livers were dissected free from gall bladder and quickly frozen in liquid nitrogen and stored at -80°C until further processing.

Extraction and analysis of retinoids. Plasma and hepatic retinoids were extracted and analysed as described by Besselink *et al.*[11].

4. Results

36 months after initiation of the experiment, plasma and liver retinoid levels were measured. The results are presented in table 1. The plasma retinol levels in flounder from the direct as well as the indirect polluted mesocosm were significantly reduced as compared to plasma retinol levels in flounder from the reference mesocosm. In addition, marked decreases in hepatic retinol concentration were observed in flounder from both the direct and indirect

Table 1
Retinoid content in plasma and liver of flounder following 36 months of exposure to either reference sediment (mesocosm 1), inderectly polluted sediment (mesocosm 2) or contaminated dredged spoil from Rotterdam harbour (mesocosm 3).

Mesocosm	plasma retinol (ng/ml plasma)	hepatic retinol (μg/g liver)	hepatic retinyl palmitate (μg/g liver)	hepatic ratio retinol/retinyl palmitate (ratio*100)
1	68.7±4.4 (27)	24.8±3.5 (14)	258.4±22.1 (14)	10.0±2.3 (14)
2	49.4±3.6** (37)	5.7±1.2** (19)	145.6±15.1* (19)	3.7±0.8** (19)
3	47.6±3.8** (21)	4.7±0.8** (14)	288.9±37.0 (14)	1.6±0.2** (14)

Number of animals in parentheses. Data are expressed as mean ± S.E..
*: significant different from mesocosm 1, $p<0.01$.
**: significant different from mesocosm 1, $p<0.001$.

contaminated mesocosm. In contrast, a slight but significant decrease in hepatic retinoid stores was found in the indirect polluted mesocosm only. This was reflected in the hepatic ratio retinol/retinyl palmitate, a measure for the mobility of hepatic retinoids, which was more affected in the direct polluted mesocosm then in the indirect contaminated mesocosm.

5. Discussion

From the present study it is clear that retinoid levels in flounder exposed for three years to harbour dredge spoil are dramatically altered. In the three mesocosms a gradient for PHAHs and PAHs was found which was reflected in flounder liver and bile[1]. In contrast a gradient for heavy metals was apparent in the sediment but not in flounder livers. This suggests that the observed changes in flounder retinoid homeostasis are a result of PHAH or PAH exposure rather than metal exposure.

In contrast to mammalian studies, data on retinoid disturbing effects of PHAHs in fish are limited and contradictory. While we did not observe marked alterations in retinoid levels in flounder after short-term exposure to either Clophen A50 or TCDD (Besselink *et al. submitted for publication)*[11], Palace and Brown[13] reported changes in retinoid levels in tissue of lake char (*Salvelinus namaycush*) eight weeks after a single oral administration of the coplanar 3,3',4,4',5-pentachlorobiphenyl. The differences in retinoid responses of fish towards PHAH exposure might be a result of the time of exposure. Gilbert *et al.*[14] reported elevated retinoic acid hydroxylation in rainbow trout (*Oncorhynchus mykiss*) 56 days after a single i.p. administration of 3,3',4,4'-tetrachlorobiphenyl (TCB), but not between TCB-treated and control groups 7 days after injection.

In conclusion, the present study clearly demonstrates that prolonged exposure of flounder to contaminated sediment results in decreased levels of plasma and liver retinol levels, which in the long term may attribute to the high incidences of diseases in flounder from contaminated estuaries and coastal areas.

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7. References

1) A.D. Vethaak, J. Jol, M.L. Eggens, A. Meijboom, T. ap Rheinallt, P.W. Wester, T. van de Zande, A. Bergman, F. Ariese, N. Dankers, F.A. Ariese, R.A. Baan, J.M. Everts, A. Opperhuizen and J.M. Marquenie (1996): Skin and liver diseases induced in flounder (*Platichthys flesus*) after long-term exposure to contaminated sediments in large-scale mesocosms. Env. Health. Perspectives, accepted.

2) A.D. Vethaak and P.W. Wester (1996): Diseases of flounder (*Platichthys flesus*) in Dutch coastal waters, with particular reference to environmental stress factors. Part 2. Liver histopathology. Dis. Aquat. Org., in press.

3) D.C. Malins, B.B. McCain, J.T. Landahl, M.S. Myers, M.M. Krahn, D.W. Brown, S.L. Chan and W.T. Roubal (1988): Neoplastic and other diseases in fish in relation to toxic chemicals: an overview. Aquat. Toxicol. 11, 43-67.

4) L.M. van Putten, D.W. van Bekkum and M.J. de Vries (1970): A severe chronic intoxication in a monkey colony. *in*: *Balner, H. & Beveridge, W.I.B. (eds.) Infections and immunosuppression in subhuman primates. Munksgaard, Copenhagen*, 155-166.

5) S.H. Safe (1994): Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit. Rev. Toxicol. 24, 1-63.

6) E.M. Silberhorn, H.P. Glauert and L.W. Robertson (1990): Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. Crit. Rev. Toxicol. 20, 440-496.

7) A. Brouwer (1987): Interference of 3,4,3',4'-tetrachlorobiphenyl in vitamin A (retinoids) metabolism: possible implications for toxicity and carcinogenicity of polchlorinated aromatic hydrocarbons. Ph.D. Thesis, University of Leiden, Leiden, The Netherlands.

8) M.B. Sporn and A.B. Roberts (1983): The role of retinoids in differentiation and carcinogenesis. Cancer Res. 43, 3034-3040.

9) S. Flodström, L. Busk, T. Kronevi and U.G. Ahlborg (1991): Modulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and phenobarbital-induced promotion of hepatocarcinogenesis in rats by the type of diet and vitamin A deficiency. Fund. Appl. Toxicol. 16, 375-391.

10) A. Brouwer, H. Håkansson, A. Kukler, K. van den Berg and U.G. Ahlborg (1989): Marked alterations in retinoid homeostasis of Sprague-Dawley rats induced by a single i.p. dose of 10 μg/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicology 58, 267-283.

11) H.T. Besselink, S. van Beusekom, R. Roex, A.D. Vethaak, J.H. Koeman and A. Brouwer (1996): Low hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) activity and minor alterations in retinoid and thyroid hormone levels in flounder (*Platichthys flesus*) exposed to the polychlorinated biphenyl (PCB) mixture, Clophen A50. Environm. Poll. in press.

12) P. Janssen, P.M.R.R. Monteiro, J.G.D. Lambert and H.J.T. Goos (1996): Spermatogenisis, testicular steroidogenesis and plasma steroid profiles inthe male flounder, *Platichthys flesus* (L.), during the annual reproductive cycle and after long-term exposure to polluted harbour sediment. pp 55-82. *in thesis: Reproduction of the flounder, Platichthys flesus (L.), in relation to environmental pollution: steroids and vitellogenesis.* University of Utrecht, the Netherlands.

13) V.P. Palace and S.B. Brown (1994): HPLC determination of tocopherol, retinol, dehydroretinol and retinyl palmitate in tissues of lake char (*Salvelinus namaycush*) exposed to coplanar 3,3',4,4',5-pentachlorobiphenyl. Environm. Toxicol. Chem. 13, 473-476.

14) N.L. Gilbert, M-J. Cloutier and P.A. Spear (1995): Retinoic acid hydroxylation in rainbow trout (*Oncorhynchus mykiss*) and the effect of a coplanar PCB, 3,3',4,4'-tetrachlorobiphenyl. Aquat. Toxicol. 32, 177-187.