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Correlation between EROD and GSH-T activities and the presence of organochlorines in the liver of dab from the Belgian continental shelf.

by

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Abstract

The ethoxyresorufin-O-deethylase (EROD) and glutathione-S-transferase (GSH-T) activities in the liver of dab (*Limanda limanda*) were correlated with the concentration of 10 chlorobiphenyl (CB) congeners (IUPAC nrs 31, 28, 52, 101, 105, 118, 138, 153, 156 and 180), hexachlorobenzene (HCB) and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE). The samples of dab were obtained from the Belgian Continental Shelf in March, May, September and December 1992. The fat content of dab liver showed a specific pattern, with the lowest concentrations in early spring and the highest concentrations in early autumn. Similar organochlorine patterns were observed in all samples and no seasonal variation was observed in the CB concentration as determined on the bassis of total extractable lipid. EROD and GSH-T activities exhibited no apparent seasonal variation. No significant correlations could be found between the concentration of the organochlorines and the EROD activities. However, in several cases a significant correlation could be found between the GSH-T activities and the organochlorine concentrations.

1. Introduction

The use of enzymatic activity as a rapid screening method in determining possible environmental pollution, has been the subject of extensive study during the last decade. Especially enzymes associated with the cytochrome .P-450 monooxygenase system such as ethoxyresorufin-O-deethylase (EROD) have proven to be well suited for this purpose (Payne et al., 1987; Jiminez and Stegeman, 1990; Goksoyr and Förlin, 1992). The monooxygenase system and conjugating enzymes are important for the detoxification processes in animals. Two types of enzymes can be distinguished: phase I enzymes e.g. EROD and phase II enzymes e.g. gluthatione transferase (GSH-T). The phase I enzymes are responsible for the addition of a more polar reactive group to exogenous products, such as apolar organic contaminants, rendering it a more

suitable substrate for phase II enzymes. The phase II enzymes create conjugates with endogenous molecules which can be more readily excreted. Several authors reported elevated EROD activities in the presence of organic pollutants such as polychlorinated biphenyls (PCB's) (Payne et al., 1987; Jiminez and Stegeman, 1990; Galgani et al., 1991; Goksoyr and Förlin, 1992). Moreover, EROD has been recommended by the Joint Monitoring Group (JMG) of the Oslo and Paris Commissions as an indicator for environmental pollution. In contrast very little attention has been payed to phase II enzymes (e.g. glutathione S-transferase (GSH-T)) as indicators of environmental pollution (Jimenez and Stegeman, 1990). Although it has been demonstrated that GSH-T activity in fish liver increases after administration of xenobiotic compounds (George and Young, 1986; Fair, 1986, Davies, 1985, Boon et al., 1992). Van Veld and Lee (1989) reported that GSH-T activity in the European flounder (*Platichtys flesus*) did not follow a pollution gradient and that the enzyme was therefore not suited as an indicator. In the present study the seasonal variations of EROD, GSH-T, hexachlorobenzene (HCB), polychlorinated biphenyls (CB's) and p,p'-dichlorodiphenyldichloroethylene were examined in the liver of dab (*Limanda limanda*) and the correlations between enzymatic activities and concentrations of organochlorines were analysed.

2. Materials and methods

2.1. Chemicals

The chlorobiphenyl (CB) congeners IUPAC nrs 28,52,101,118,138,153 and 180 were certified reference materials purchased from the Community Bureau of Reference (BCR).

The chlorobiphenyl congeners IUPAC nrs 31,105 and 156, p,p'-dichlorodiphenyldichloroethylene (p,p' -DDE) and hexachlorobenzene (HCB) were purchased as pure isomers (Promochem, Wesel).

Ethoxyresorufin was synthesized according to the method of Prough et al. (1977), modified by Klotz et al. (1984). Purity was controlled by thin-layer and reversed phase liquid chromatography with ethoxyresorufin from the Sigma chemical company as a reference.

All others chemicals were of research grade quality.

2.2. Sampling

The samples of dab were obtained from the Belgian Continental Shelf in March, May, September and December 1992 during scientific cruises of the oceanographic vessel Belgica. At least 12 samples of dab liver per period were collected for chemical and biochemical analysis. Although sampling occured on several locations, only one site (site n° 120 (Westdiep) as is described in Cooreman et al., 1993) was used for correlation analysis between chemical and biochemical data.

2.3. Biochemical analysis

EROD and GSH-T activities were measured onboard in freshly collected fish livers. The EROD activity was fluorimetrically determined, according to a modification of the method of Burke and Mayer (1974). The GSH-T activity was spectrophotometrically determined according to a modified method of Warholm et al. (1985). More detailed information on the biochemical analysis can be found in Cooreman et al. (1993). The livers were subsequently stored by freezing (-20 °c) prior to chemical analysis.

2.4. Chemical analysis

2.4.1. Extraction and clean-up

Extraction was based on the total lipid extraction of Bligh and Dyer (1959). The lipid material that was used for the determination of the fat content was redissolved in hexane (see also Vandamme (1982)) and cleaned on a florisil column (deactivated during 16 hours at 420 °C and activated with 1.5 % water). The organochlorines were subsequently eluted with hexane. After addition of the internal standard tetrachloronaphtalene (TCN) and isooctane as a keeper, the hexane fraction was concentrated in a rotary evaporator and further under a stream of nitrogen to 1 ml. This solution was analysed by gaschromatography. Quality control was assured using certified reference materials (CRM 349, BCR) and participation in international intercalibration exercises (ICES/OSPARCOM intercalibration for CB's in marine media).

2.4.2. Chromatographic conditions

Instrument: CE 8160

Column: J&W DB 17, lenght: 60 m, internal diameter: 0.25 mm, film thickness: 0.25 μm.

Carrier gas: Helium, 150 kPa

Injection: 1 μl splitless, splitter open 1 min 10 after injection.

Temperatures: Injector: 235 °C, Detector: 310 C, Oven program: initial temperature: 90 °C for 2 min, 1st rate: 15 C/min to 150, 2nd rate: 3 C/min to 265 °C, Isothermal: 10 min, 3th rate: 15 C/min to 275 C, Isothermal: 10 min.

Detection: Ni 63 electron capture detector.

3. Results

3.1. Seasonal variation of fat.

The results of the fat analysis can be found in table 1. The fat content of the liver showed a specific pattern, reaching its lowest concentration in early spring, steadily rising towards a peak concentration in late summer and early autumn and decreasing again during winter (figure 1).

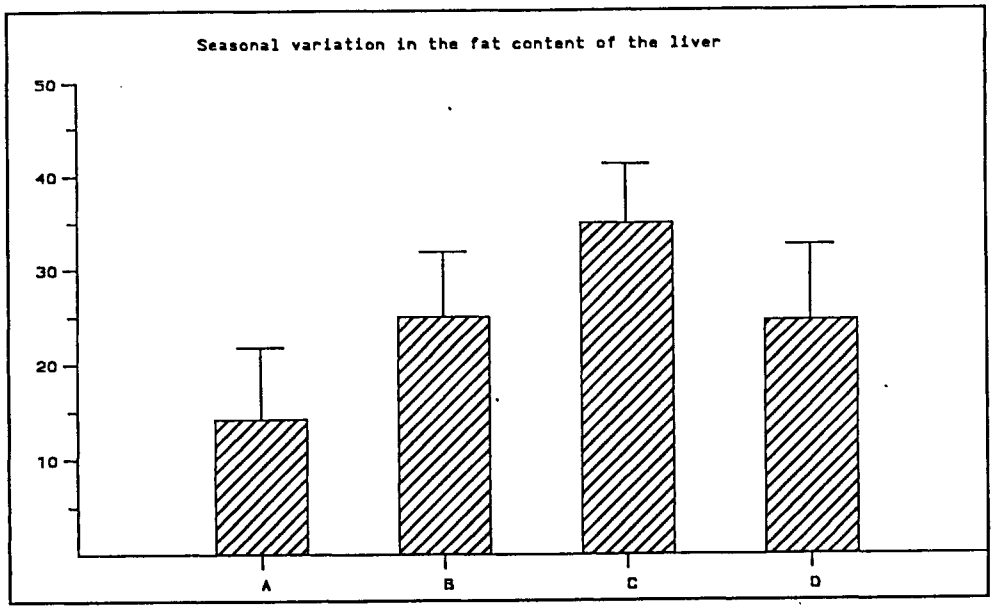

Figure 1. Seasonal variation of the fat content in dab liver. The X axis represents the sampling periods (A=March, B=May, C=September and D= December). The Y axis represents the fat content of the liver expressed as % fat (mean and standard deviation).

3.2. Seasonal variation of CB's, HCB and p,p'-DDE.

The results of the chemical analyses can be found in table 1. The concentration varied between 209 ng/g extractable lipid and 899 ng/g extractable lipid for the CB's (expressed as the sum of the CB's nrs 28, 31, 56, 101, 105, 118, 138, 153, 156 and 180), 3 ng/g extractable lipid and 35 ng/g extractable lipid for HCB and between 15 ng/g extractable lipid and 61 ng/g extractable lipid for p,p'-DDE. Among the CB congeners nrs 153 and 138 were always present in the largest concentrations. Moreover, the organochlorine patterns (figure 2) of the different samples appeared to be constant.

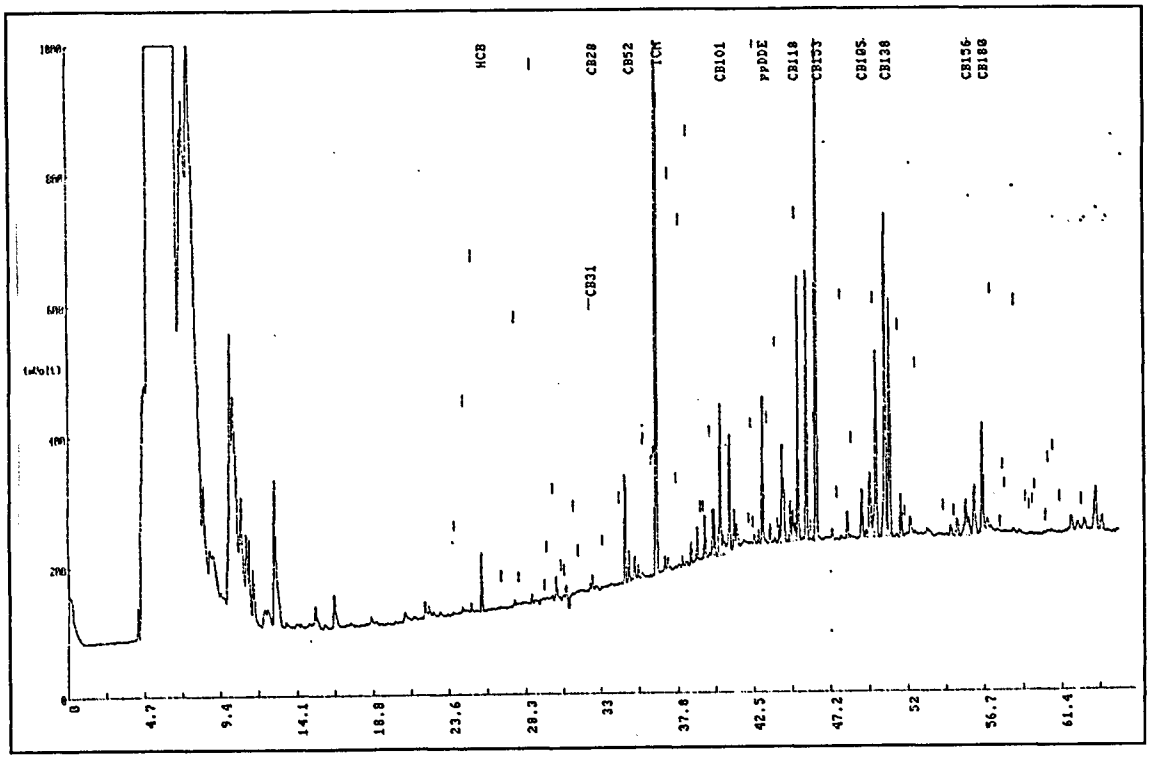

Figure 2. Chromatogram illustrating the typical CB pattern that was found in the liver of dab (*Limanda limanda*).

Further, no seasonal variation appeared in the CB concentration as determined on the basis of total extractable lipids (figure 3). This was statistically confirmed using an ANOVA test (P = 0.8712).

Plotting the total CB content of the liver (calculated on the basis of the fat content of the liver and the CB concentration of the fat, see table 1) against the period of sam-

pling (figure 4) resulted in an entirely different picture. As with the seasonal variation of the fat content (figure 1), the total CB concentration increased during spring and summer, reached a peak concentration in late summer and early autumn, decreased again during wintern and reached the lowest concentration in early spring.

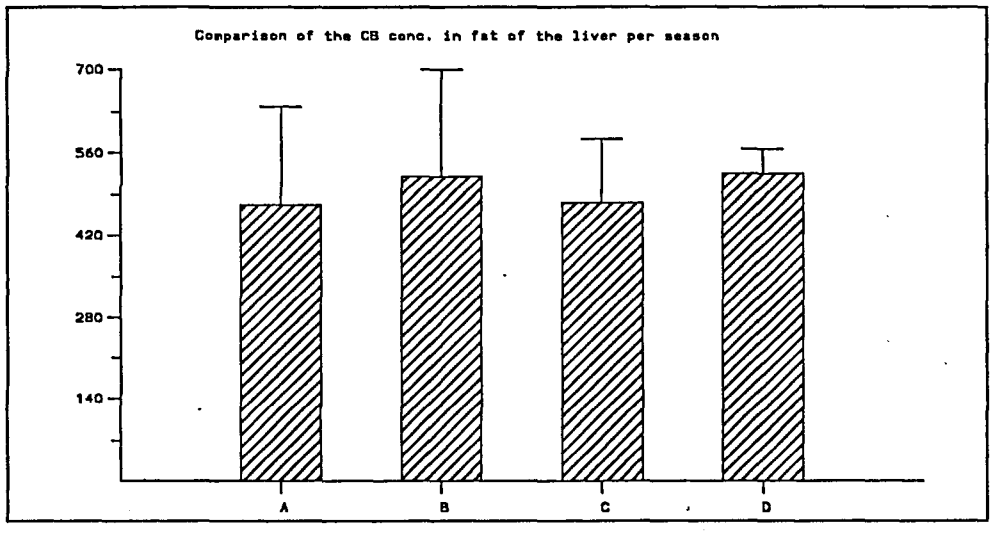

Figure 3. Seasonal variation of the CB concentration in dab liver. The X axis represents the sampling periods (A=March, B=May, C=September and D= Decémber). The Y axis represents the CB concentration, expressed as ng/g extractable lipid (mean and standard deviation).

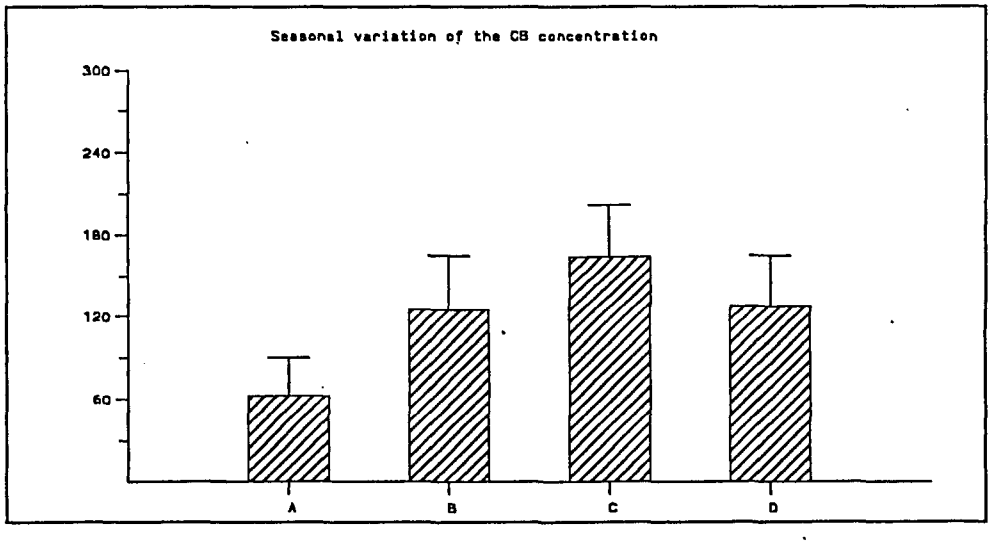

Figure 5. Seasonal variation of the CB concentration in dab liver. The X axis represents the sampling periods (A=March, B=May, C=September and D= Decémber). The Y axis represents the CB concentration, expressed as ng/g total liver weight (mean and standard deviation).

TABLE 1

Results of the chemical and biochemical analyses for the different periods.

Period	Sample nr.	EROD activity *** pm/mg.min	GSH-T avtivity **** nm/mg.min	SUM CB ** ng/g	HCB ** ng/g	p,p'-DDE ** ng/g	% Fat in Liver
March '92	1	3639	57	712	13	48	16.3
	2	1933	146	628	14	50	14.7
	3	3757	98	544	14	35	14.9
	4	382	91	273	5	28	20.1
	5	850	467	352	9	31	7.1
	6	737	214	209	8	16	33.6
	7	156	114	423	8	27	18.5
	8	626	301	673	35	53	8.5
	9	120	153	565	21	47	12.6
	10	2166	231	430	13	30	8.0
	11	967	256	280	8	30	7.8
	12	210	298	570	12	38	8.5
May '92	1	107	127	367	5	21	26.8
	2	11	89	357	8	15	12.8
	3	* n.d.	342	630	13	46	27.5
	4	* n.d.	296	647	18	50	22.6
	5	* n.d.	226	379	10	42	28.3
	6	12	229	899	13	61	21.8
	7	* n.d.	208	763	11	48	14.7
	8	* n.d.	167	471	10	27	32.6
	9	* n.d.	88	350	6	15	37.8
	10	* n.d.	178	403	10	35	23.9
	11	* n.d.	171	423	15	24	27.6
	12	* n.d.	247	549	9	28	22.9
September '92	1	* n.d.	213	640	5	45	24.5
	2	* n.d.	207	579	6	30	37.8
	3	59	443	528	8	37	29.6
	4	* n.d.	195	379	6	20	33.0
	5	* n.d.	250	374	3	42	44.1
	6	* n.d.	260	458	4	21	41.3
	7	38	266	649	10	46	33.3
	8	* n.d.	399	430	5	21	27.9
	9	* n.d.	228	419	4	18	44.4
	10	59	278	323	3	17	39.6
	11	* n.d.	328	440	4	20	31.1
	12	* n.d.	203	382	5	20	30.6
	13	26	417	584	5	30	36.6
December '92	1	* n.d.	*n.a.	521	9	42	29.6
	2	* n.d.	*n.a.	503	8	46	33.4
	3	30	*n.a.	615	9	62	15.5
	4	* n.d.	*n.a.	527	6	32	25.5
	5	* n.d.	*n.a.	502	7	56	15.2
	6	38	*n.a.	494	7	56	33.6
	7	* n.d.	*n.a.	506	5	88	19.0

* n.d.= not detectable; n.a.= not analysed

** ng/g is expressed as ng per g extractable lipid

*** pm/mg.min is expressed as picomoles resorufin production per minute and per mg protein

**** nm/mg.min is expressed as nanomoles S-2,4-dinitrophenylglutathione production per minute and per mg protein

3.3. Seasonal variation of EROD and GSH-T activities

The results of the biochemical data can be found in table 1. The results for EROD have been extensively discussed by Cooreman et al. (1993). Enhanced EROD activities were observed in March, with considerable differences between the individual activities. During the other sampling periods no or very little activity was observed. The GSH-T activities showed little difference for the three periods, with the exception of slightly elevated activities in September.

3.4. Correlation analysis between chemical and biochemical data.

3.4.1. EROD activity vs organochlorine concentrations.

EROD activities were plotted against the concentration of organochlorines (expressed as ng/g liver) and the results were statistically analysed for correlation (Pearson correlation test). The correlation coefficient (Pearson r) did not exceed 0.47 in any case and which would suggest that there is no significant correlation between the EROD activity and the concentration of the organochlorines.

3.4.2. GSH-T activity vs organochlorine concentrations.

GSH-T activities were plotted against organochlorine concentrations (expressed as ng/g liver) and the results were statistically analysed for correlation (Pearson correlation test). The results (table 2) suggested a negative linear correlation between GSH-T and total CB's (r=-0.75) and GSH-T and p,p'-DDE (r=-0.77) in March (fig.5) and a positive linear correlation between GSH-T and p,p'-DDE (r=0.78), GSH-T and total CB (r=0.61) and GSH-T and HCB (r=-0.58) in May (fig.6). When comparing the data for September, no correlation was obtained. Unfortunately, no data were available for December as was the case with EROD.

TABLE 2

Results of the correlation analysis for GSH-T and organochlorines

Date	Comparison	* r
March '92	GSH-T vs Sum CB	-0.75
	GSH-T vs HCB	-0.32
	GSH-T vs p,p' DDE	-0.77
May '92	GSH-T vs Sum CB	0.61
	GSH-T vs HCB	0.58
	GSH-T vs p,p' DDE	0.78
September '92	GSH-T vs Sum CB	0.00
	GSH-T vs HCB	0.10
	GSH-T vs p,p' DDE	-0.03

* Pearson correlation coeffficient

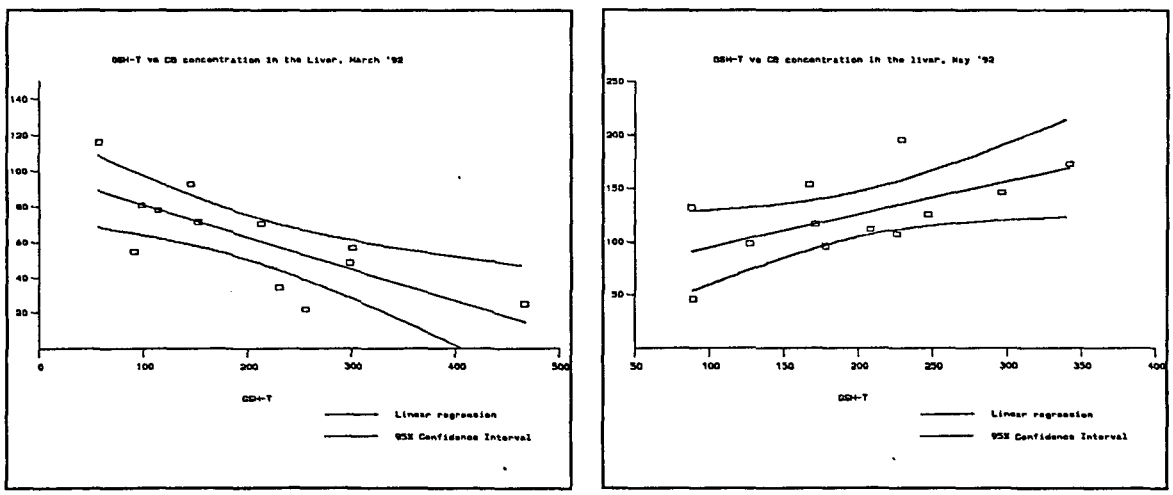

Figure 5. GSH-T activity plotted against the concentration of total CB in March (left) and May (right). The X axis represents the GSH-T activity, expressed as nanomoles S-2,4-dinitrophenolglutathione production per minute and per mg protein. The Y axis represents the organochlorine concentration, expressed as ng per g liver.

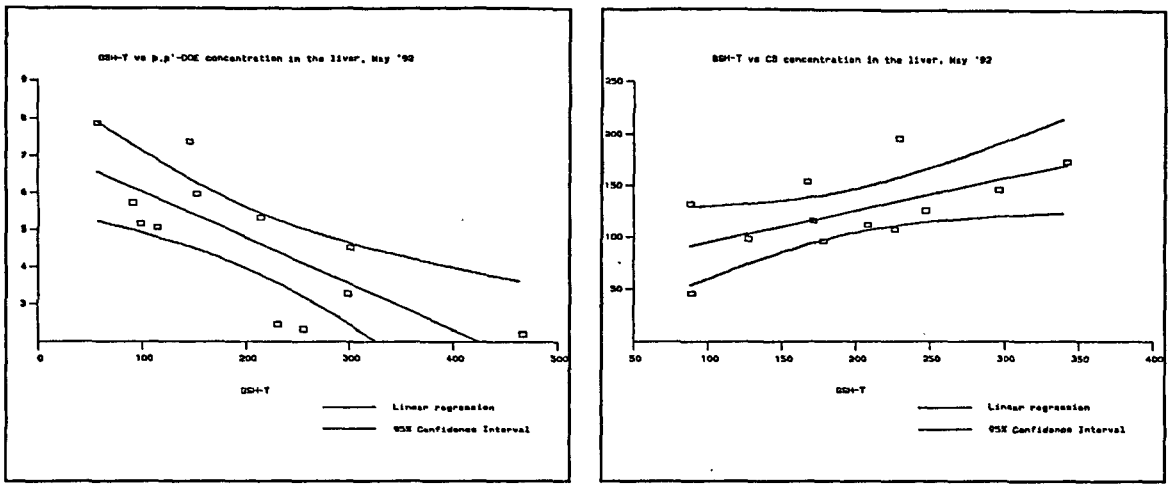

Figure 6. GSH-T activity plotted against the concentration of p,p'-DDE in March (left) and May (right). The X axis represents the GSH-T activity, expressed as nanomoles S-2,4-dinitrophenolglutathione production per minute and per mg protein. The Y axis represents the organochlorine concentration, expressed as ng per g liver.

4. Discussion

The concentration levels of the organochlorines were similar to those reported by Knickmeyer and Steinhart (1990). Fig.3 shows that the CB concentration in the fat of dab liver remained constant during the year. The hepatic fat concentration however decreased two and a half fold between September and March (fig.1). This implies that the hepatic CB concentration normalised to the wetweight of the liver varies in a constant ratio to the fat content (fig.4). Maximum values for the total CB concentration per mg wet weight were found in September. Towards March the CB concentration decreased to approx. 50 ng/g wetweight. These results clearly indicate that the seasonal variation of the CB content in the liver is related to the variation of the fat content in the liver. The decrease of fat in the coldest period of the year is most probably caused by the changing metabolic status of the animal. To compensate for food shortages fat is metabolised. The joint variation of fat and CB content points to a state of equilibrium between the CB concentration in the fat of the liver and other parts of the animal and maybe particulate organic matter outside the animal. Equilibrium partitioning of CBs between body lipids, blood and the ambient water was reported by Duinker and Boon (1985) as the dominating process regulating the CB

content in gill-breathing animals. The elimination of CBs and presumably other chemicals out of the liver during fat metabolism could be responsible for the EROD induction during winter. A significant increase in EROD activity was observed in the coldest period of the year (Cooreman et al., 1993). The seasonal variation of EROD seems to be inversely related to the variation of CB and fat. On the other hand an increase in EROD activity could be expected in December when the fat and CB content began to decrease. However no induction was observed. The decrease of EROD and simultaneous increase of hepatic CB and fat concentrations in summer gives room to the suggestion that the CBs in the fat are well isolated and not available to cause induction of the defence systems. Although similar results were expected for HCB and p,p'-DDE, this could not be demonstated with the results at hand. Contrary to previous results reported in literature (Galgani et al., 1991), no correlation could be demonstrated between EROD activities and the concentration of organochlorines.

The seasonal variations of the correlation coefficients between GSH-T and total CBs and between GSH-T and p,p'-DDE may refer to complex biochemical processes. The respective decrease and increase in enzyme activity with increasing organochlorine concentration in March and May points to enzyme inhibition and induction (fig.5 & fig.6). Whether the inhibition is caused by the compounds examined or by other contaminants is not clear. GSH-T induction by PCBs in fish was previously reported (Andersson et al., 1985 ; Boon et al., 1992). Based on these results an inhibitory role for CBs on GSH-T should be considered as unlikely to occur. This could imply that the correlation found between CBs and GSH-T was established by coincidence. Thomas et al. (1982) and Thomas and Wofford (1984) observed elevated lipid peroxidation in liver homogenates and microsomes from mullet (Mugil cephalus) and Atlantic croacker (Micropogonias undulatus) exposed to a PCB mixture. According to our results, CB's are liberated from liver fat in March, which may enhance peroxide production. Experiments with mussels performed by Viarengo et al. (1991) showed clear seasonal variations with enhanced oxidative stress in mussels accompanied by a decrease in the concentration of peroxide scavengers (e.g. glutathion, vitamin E, others) and antioxidant enzyme activities in winter. In May an inversed pattern was observed. Based on these results two mechanisms could influence the level of GSH-T

activities in dab liver : variation of the glutathion content and inhibition by peroxides.

The above considerations and the presence of a correlation between GSH-T and the organpchlorines examined, stress the need for further research on the toxicokinetical mechanisms that influence the cellular metabolism.

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