Molecular Responses as Indicators of Marine Pollution: DNA Damage and Enzyme Induction in *Limanda limanda* and *Asterias rubens*

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During a survey from 26 August through 13 September 1991, specimens of the flatfish, *Limanda limanda* (dab), and the asteroid echinoderm, *Asterias rubens* (seastar), were collected at sampling locations along transects radiating into the North Sea from the coastal zone of The Netherlands. In homogenates of liver tissue from male dab and the digestive gland (pyloric caeca) of female seastar, DNA damage (strand breaks) and induction of the cytochrome P450-dependent monoxygenase system (MO) were determined. Areas could be described with significantly increased percentages of strand breaks (lower integrity) both in dab and seastar. However, enhanced DNA strand breaks did not correspond with contamination gradients, expressed as concentrations of polychlorinated biphenyls (PCBs) or polyaromatic hydrocarbons. MO enzyme induction in the hepatic 13,000g fraction of male dab, measured as 7-ethoxyresorufin-O-deethylase activity, was significantly enhanced in response to low ambient temperatures. Some evidence was found for the facilitation of benzo[a]pyrene hydroxylase activity expressing the enzyme induction in the microsomal fraction of pyloric caeca of seastars, at increasing PCB concentrations. DNA integrity and enzyme induction elucidate the physiologic status and might be indicative for ambient impairment within restricted areas, and not necessarily related to the presence of anthropogenic or xenobiotic substances. — Environ Health Perspect 102(Suppl 12):37-43 (1994)

Key words: molecular response, biomarkers, dab, seastar, DNA strand breaks, cytochrome P450, EROD activity, BPH activity, marine pollution

Introduction

The anthropogenic contaminant load in the world's marine systems induces detrimental environmental conditions, affecting the biologic integrity of ecosystems as well as physiologic functions of individual organisms. In particular, biologically critical estuarine and coastal systems are exposed most intensively to a high degree of contamination. In addition, there is hardly any region in the open ocean which is not influenced by contaminants, either of anthropogenic origin (predominantly halogenated hydrocarbons) or natural chemical substances at elevated concentrations (heavy metals and polyaromatic hydrocarbons). The occurrence of many of these compounds in all marine environmental compartments (water, suspended particulate matter, sediment, and biota) is well documented (1).

To assess the quality of the (marine) environment, it is important to evaluate the impact of chemical substances in terms of molecular responses reflecting the potential for impairment of physiologic and biologic processes in exposed organisms. With respect to the application of toxicologic effects for the assessment of environmental health, research on biologic markers (or biomarkers) is most relevant, since it includes the determination of the physiologic status of organisms. However, the ecologic significance of biomarkers has remained largely undocumented until now. Biomarkers have been defined in many ways, and the implementation of biomarker-based studies in ecotoxicologic research and environmental monitoring programs increases more and more (2,3). A biologic marker can be defined as a change in a biologic response measured in biologic systems ranging from molecular through biochemical, cellular, and physiologic responses to behavioral changes, and which can be related to an exposure to, or toxic effect of, an environmental chemical or chemicals. The strategy for biomarker research and the application, implementation, and validation of biomarker-based studies in the assessment of environmental health and in ecotoxicologic studies have been discussed extensively (3).

In the context presented here, biologic markers will be defined as measurements of responses of molecules (DNA and two cytochrome P450-dependent enzymes), that indicate the presence and magnitude of toxicants in molecular or biochemical terms (integrity of genetic material and induction of biotransformation enzymes, respectively).

According to a hypothesis for pollutant monitoring in the context of occupational (human) health (4), early departures from health are accompanied by little disability. In such a situation of preliminary impairment, normal homeostatic processes insure adequate adjustment to offset stress. Subsequently, under conditions of ongoing impairment, compensatory processes similarly maintain the overall function of the system, without serious disability. During this compensation phase with normal body functions being maintained, however, there may already be a transgression of the state of homeostasis. Further
increments of impairment beyond the limit of compensation into a noncompensatory phase, might cause disturbed functions, overt diseases and ultimately death. Depledge (5) considered that, theoretically, the model described by Hatch (4) and modified by Jager (6) is analogous to that facing marine ecotoxicologists today. Thus, the hypothesis based essentially on departures from normal physiologic and behavioral responses due to impairment, can be applied to systems of various integration levels, from molecule to organism or population. Early departures from homeostasis (healthy state of the system) are associated with the initiation of compensatory responses, which are distinct from normal homeostatic responses. In ecotoxicology, it is essential to be able to identify these compensatory responses in order to assess the health status of the environment. These responses might occur at the molecular level initially and lead to deleterious effects at a higher organizational level.

The objectives of the recent ecotoxicological research carried out in the Department of Chemical Oceanography and Marine Pollution of The Netherlands Institute for Sea Research focuses on the development and application of selected molecular and biochemical responses in a number of marine animal species, to estimate the status of the system with respect to homeostasis or a compensation phase or to predict possible departures from health. Two biomarkers are involved in the present research:

**Genetic Damage**

Exposure to contaminants may lead to genetic alterations. Damage of genetic material may function as a biologic marker and this can include measures of damage from specific chemical agents (e.g., BaP–DNA adduct formation) or can be nonspecific indicators of damage to the integrity of DNA (e.g., number of strand breaks in the DNA). Damage to DNA has been proposed as a useful parameter for assessing the genotoxic properties of environmental contaminants (7–9).

**Induction of Detoxifying Systems**

Many contaminants stimulate specific detoxification mechanisms, such as the cytochrome P450-dependent monoxygenase (MO) enzyme system. Higher enzyme levels or activity is evidence of a molecular response to toxicant exposure. In this system, cytochrome P4501A plays a central role in the metabolism of a variety of xenobiotics (such as PCBs or polycyclic aromatic hydrocarbons [PAHs]) and has been found to be a very sensitive biomarker in aquatic animal species (10,11).

The aim of the present study was to describe the quality of the coastal and offshore area of the southern part of the North Sea in terms of molecular and biochemical responses. The present study investigated DNA integrity and the induction of the cytochrome P450-dependent MO enzyme system in two marine animal species, collected from their natural habitat. DNA integrity, expressed as the percentage double-strandedness, was determined in liver and the digestive gland (pyloric caeca) of the bottom dwelling fish, dab (Limanda limanda L.) and the macrobenthic echinoderm, seastar (Asterias rubens L.), respectively. The induction of the cytochrome P4501A gene (CYP1A), was measured catalytically as 7-ethoxyresorufin-O-deethylase (EROD) activity in dab and as benz[a]pyrene hydroxylase (BPH) activity in seastar.

**Methodology**

Samples were obtained during a survey with the research vessel *Pelagia* in the Southern Bight and the Central North Sea from 26 August through 13 September, 1991. Male dab and seastars were collected by means of beam trawling (trawling time 15 min) at sampling locations along transects radiating into the southern part of the North Sea from coastal areas of The Netherlands (Figure 1). The area of study reflected a gradient from estuarine and coastal zones, considered to be highly contaminated with polychlorinated biphenyls (PCBs) and/or polycyclic aromatic hydrocarbons (PAHs) (stations 10, 11, 14, 17) to (expected) pristine open-sea reference sites (stations 4, 5, 6). Details on the treatment of the samples and sample preparation for biochemical and chemical analysis are described elsewhere (12,13).

Strand breaks in DNA isolated either from liver tissue of dabs or from the pyloric caeca of seastars were measured by an alkaline unwinding assay (14). To assess the level of strand breaks in aquatic species the alkaline unwinding assay was modified to allow for the isolation of intact highly polymerized DNA and the subsequent strand breaks (15). The technique is based on the time-dependent partial alkaline unwinding of DNA followed by determination of the [double:total] DNA ratio (F-value).

The assays for the determination of EROD in dab liver were performed according to the procedures described previously (16), and were carried out in samples diluted with 0.1% albumin in 0.1 M phosphate buffer, pH 7.4, containing 0.5 μM of 7-ethoxyresorufin and 0.15 mM NADPH. The method is based on the fluorometric determination of EROD activity in the 13,000g supernatant fraction of liver tissue homogenates, and expressed as

![Figure 1. Sampling locations during a survey in the southern and central part of the North Sea (26 August–13 September 1991).](image-url)
Table 1. Sum concentration (Σ4CB in ng/g lipid) of four chlorinated biphenyl congeners (CB101, -118, -153 and -105) in muscle tissue of Limanda limanda (dab) and in the pyloric caeca of Asterias rubens (seastar).

| Station number | Σ4CB in seastar (ng/g lipid) | Σ4CB in dab (ng/ml) | Σ24PAH in sediment (μg/g) | 1-0H-pyr (μg/l) in dab bile (mean ± SD) | F-value (± CI, n = 10) in seastar (15) | F-value (± CI, n = 10) in dab (15) | BPH (μg/ml) in seastar (15) | ERD (μg/ml) in dab (15) |
|----------------|-----------------------------|---------------------|-------------------------|----------------------------------------|----------------------------------------|----------------------------------------|---------------------------|--------------------------|
| 2              | 250.1                       | 339.4               | 54                      | 130 ± 60                                | 0.684 ± 0.068                          | 0.834 ± 0.057                          | 10.4 ± 2.5                | 0.057 ± 0.035             |
| 3              | 255.0                       | 322.1               | 88                      | 50 ± 20                                 | 0.517 ± 0.044                          | 0.744 ± 0.056                          | -                         | 0.038 ± 0.031             |
| 4              | 200.3                       | 345.4               | 275                     | 40 ± 20                                 | 0.423 ± 0.054                          | 0.775 ± 0.067                          | 0.032 ± 0.022             | 0.763 ± 0.032             |
| 5              | 48.3                        | 235.0               | 42                      | -10                                     | 0.765 ± 0.105                          | 0.908 ± 0.041                          | 20.0 ± 0.2                | 0.109 ± 0.093             |
| 6              | No seastar                  | 260.8               | 82                      | 20 ± 10                                 | -                                       | -                                      | -                         | -                        |
| 7              | 236.6                       | 333.3               | 115                     | 150 ± 60                                | 0.651 ± 0.116                          | 0.887 ± 0.040                          | -                         | -                        |
| 8              | 138.0                       | 263.7               | 440                     | 60 ± 10                                 | 0.378 ± 0.086                          | 0.826 ± 0.054                          | 0.116 ± 0.068             | 0.930 ± 0.049             |
| 9              | 177.7                       | 375.8               | 52                      | 20 ± 10                                 | 0.530 ± 0.116                          | 0.751 ± 0.070                          | -                         | 0.066 ± 0.049             |
| 10             | 432.1                       | 350.0               | 71                      | -                                       | 0.402 ± 0.061                          | 0.802 ± 0.299                          | 0.147 ± 0.064             | -                        |
| 11             | 376.3                       | 498.9               | 48                      | 170 ± 70                                | 0.479 ± 0.075                          | 0.762 ± 0.054                          | 0.240 ± 0.137             | -                        |
| 12             | 479.4                       | 364.0               | 37                      | 20 ± 20                                 | 0.483 ± 0.105                          | 0.770 ± 0.040                          | 0.110 ± 0.063             | -                        |
| 13             | 313.1                       | 356.7               | 38                      | -                                       | 0.578 ± 0.098                          | 0.750 ± 0.097                          | 0.142 ± 0.084             | -                        |
| 14             | 358.5                       | 339.9               | 50                      | 70 ± 60                                 | 0.645 ± 0.091                          | 0.771 ± 0.051                          | 12.8 ± 3.9                | 0.045 ± 0.029             |
| 15             | 295.9                       | 364.4               | 37                      | -                                       | 0.741 ± 0.102                          | 0.787 ± 0.061                          | 0.136 ± 0.060             | -                        |
| 16             | 105.3                       | No dab               | 28                      | No dab                                  | 0.798 ± 0.150                          | No dab                                 | 10.2 ± 1.6                | -                        |
| 17             | 412.3                       | 515.8               | 116                     | 50 ± 60                                 | 0.506 ± 0.079                          | 0.777 ± 0.054                          | 12.1 ± 0.7                | 0.065 ± 0.039             |

Abbreviations: Σ4CB, chlorinated biphenyls sum concentration of four CB congeners; Σ24PAH, sum concentration of 24 polyaromatic hydrocarbons; 1-0H-pyr, sum concentration of 1-hydroxyphenone; BPH, benzo[a]pyrene hydroxylase; ERD, ethoxyresorufin-O-deethylase. *Sum concentration (Σ24PAH in ng/g dry weight) of 24 polyaromatic hydrocarbons in bulk surface sediment (23) and the concentration of (1-0H-pyr, expressed as the arithmetic mean ± standard deviation, in ng/ml in bile of dab (24), DNA integrity (F-value, expressing the fraction double-stranded DNA ± 95% confidence interval (N-1 degrees of freedom), and enzyme induction (BPH activity in pmol/min/mg protein ± SD), in seastar and dab. No data available; the concentration of 1-0H-pyr in bile from other flatfish was high: 240 and 120 ng/ml in flounder and plaice, respectively (24).

nnmols of resorufin released per minute per milligram protein.

Benzo[a]pyrene hydroxylase (BPH) activity in pyloric caeca microsomes of seastar was assayed fluorometrically as described previously (17), detecting primarily phenols only.

Selected polychlorinated biphenyl congeners were analyzed in muscle tissue of dab (since all liver tissue had to be used for biochemical analyses) and in the pyloric caeca of seastar. The analyses of PCBs in muscle tissue as a model for the qualitative changes in the entire fish appears legitimate, because lipid-based PCB concentrations vary only slightly between different tissues of the same animal and relative changes between sampling locations are similar (18), even though absolute concentrations differ between organs and tissues. Analyses of the samples were performed using a temperature-programmed gas chromatograph, equipped with a fused silica capillary column (CP-Sil8, 50 m length, 0.15 mm bore, 0.30 μm wall coating) and 63Ni-electron capture detection (ECD). Further details on the procedure for sample extraction, clean-up, fractional separation of most pesticides and PCBs, the gas-chromatographic analysis, and the interpretation of chromatograms with respect to the identification and quantification of individual chlorinated biphenyl (CB) congeners is documented elsewhere (12,19).

All analytical steps and procedures were performed according to formalized Standard Operating Procedures and placed in the framework of intercalibration exercises of the International Council for Exploration of the Sea on PCPs, and the EC program QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe).  

Results and Discussion

The sampling stations were characterized by the degree of contamination expressed as the sum concentration of four CB congeners (Σ4CB), out of the many analyzed. These congeners are the diortho CB101 (2',2',4,5,5'-pentachlorobiphenyl), the mono-ortho CB118 (2,3',4,4',5-pentachlorobiphenyl), the diortho CB153 (2',2',4,4',5,5'-hexachlorobiphenyl) and the monoortho CB105 (2,3',4,4'-pentachlorobiphenyl), and were selected because their environmental occurrence is well established and they show well-separated noncoeluting peaks in the chromatogram. The congeners represent two levels of overall chlorination and differ in the site of the chloro-atom substitution on the biphenyl skeleton. They are given here in sequence of elution in the chromatogram and numbered according to IUPAC rules, as suggested by Ballschmiter and Zell (20).

Distinct concentration gradients in the North Sea, along transects perpendicular to the Dutch coast, were described for PCPs and PAHs in water, sediment, and organisms (21). Such gradients were also described for the concentration of individual CB congeners in dab and in seastars (12,22). The data on the sum concentration of the congeners CB101, -118, -153 and -105 (Σ4CB), considered in the present study (Table 1) are shown in Figure 2. It is generally accepted that, for example, PCB concentrations represent contamination gradients. However, these gradients, expressed in terms of concentration levels of chemicals, show restricted value with respect to associations with toxicological effects. Since PCBs, in particular certain
nonortho and monoorth CB congeners are known to be inducers of the cytochrome P450-dependent MO system (10,11) and PAHs, such as benzo[a]pyrene (BaP) induce genetic damage (strand breaks), it could be expected that gradients of these molecular responses could be described as well. However, no statistically significant (at the p<0.05 level, linear regression) correlation was found between Σ4CB and DNA integrity in dab (Figure 3a) and seastar (Figure 3c), nor between Σ4CB and the EROD and BPH activity in dab (Figure 3b) and seastar (Figure 3d), respectively. Also, no significant correlation (at the p<0.05 level, linear regression) was found between these several responses in dab and seastar and the sum concentration of 24 polycyclic aromatic hydrocarbons (Σ24PAH) in bulk surface sediment. The Σ24PAH concentrations (Table 1) were derived from data of Klungsøy and Wilhelmsen (23), and the compounds considered included a.o. naphthalenes, anthracenes and pyrenes (such as BaP). In dab, no correlation was found between BaP, measured as its metabolite 1-hydroxy-pyrene (1-OH-pyr), level in bile [data derived from Ariese (24); Table 1], and both the DNA integrity and enzyme induction.

Considering the data carefully (Table 1; Figure 3), groups of stations could be described on the basis of aberrant F-values and EROD or BPH activity. With respect to DNA integrity in dab (Figure 3a), the stations could be grouped into three clusters: low Σ4CB concentration, high integrity (high F-value); low to intermediate Σ4CB concentration, low to intermediate F-values; and high Σ4CB concentrations, low F-value. DNA integrity in seastars could be distinguished into four clusters (Figure 3c). At corresponding low Σ4CB concentrations the DNA demonstrated either high (stations 5, 16) or low (stations 4, 8) integrity. Low F-values were also measured at high Σ4CB concentrations (stations 10, 11, 12, 17). In between, a group of stations with both intermediate CB concentrations and F-values could be described. Part of the results could be explained when considering the concentration of Σ24PAH in the sediment (23) and the concentration of

Figure 3. Molecular responses in dab (Limanda limanda L.) and seastar (Asterias rubens L.): DNA integrity (F-value, expressing the fraction double-stranded DNA) in liver tissue of dab (a) and pyloric caeca of seastar (c) induction of cytochrome P450-dependent monooxygenase systems, expressed as EROD activity (nmole min/mg protein) in the 13,000g fraction of liver tissue of dab (b) and as BPH activity (pmole min/g wet weight) in the microsomal fraction of pyloric caeca of seastar (d).
1-OH-pyr in the bile of dab (24). Reduced DNA integrity (0.7 < F < 0.8) in dab liver tissue was related either to enhanced concentrations of PAHs in the bulk sediment or 1-OH-pyr in the bile (Figure 3a; stations 3, 4, 10, 11, 12). Pronounced high DNA integrity (F ≥ 0.9) was found in dab from the reference sites (stations 5, 6), with low Σ4CB (235–260 ng/g lipid) in muscle tissue, relatively low PAHs (42–82 ng/g dry weight) in sediment and a low 1-OH-pyr concentration (10–20 ng/ml) in bile (Table 1). Also, the DNA integrity in dab from station 7 was high (F = 0.87), at little enhanced Σ4CB concentration and low Σ24PAH concentration in sediment and in spite of a high 1-OH-pyr concentration (Table 1). However, the DNA integrity in dab from any particular station could not be explained by any one of these parameters.

The EROD activity in the hepatic 13,000 g supernatant fraction of dab sampled at the off-shore stations 4, 6, and 7 (Figure 3b) was significantly higher (p < 0.05) than in all other, both coastal and off-shore stations (13). To test for differences in EROD activity between the sampling locations, statistical analyses were performed on log-transformed data using a Least Significant Difference Bands test and Hochberg’s GT2 test for multiple comparisons of means (25). The data on EROD activity were confirmed by the results obtained from the semiquantitative determination of CYP1A protein levels (13). Within this group of stations no significant enzyme induction (low EROD activity) was found in relation with Σ4CB concentration. Only EROD activity at coastal station 11, showing a high concentration of the several CB congeners, was significantly higher (p < 0.05) than at other coastal or off-shore stations in the southern North Sea. This might be an indication for a possible influence of CB congeners inducing enzyme activity. This idea is supported by the most recent data obtained from a second survey in the North Sea (1992) by Sleiderink (unpublished data), describing enhanced EROD activities in relation with CB congeners, and measured within a group of 25 male dab, formed as five specimens from five hauls, from each sampling site. In the present study, exceptionally high EROD activity was found in animals obtained from stations showing stratification of the water column, resulting in low bottom water temperatures of 9.5 to 12.5°C. Bottom water temperatures at other stations varied between 17 and 20°C. A distinct effect of temperature on CYP1A induction in dab was found in field studies in the southern North Sea and in laboratory experiments (13).

Seastars showed a high DNA integrity in areas (stations 5, 16) with the lowest concentrations of both Σ4PCB and Σ24PAH (Figure 3c). Sampling locations, in particular stations 4 and 8, showing low DNA integrity (F = 0.4) had high concentrations (270–420 ng/g dry weight) of PAHs in sediment. In seastars from the coastal zone (stations 10, 11, 12, 17) with high Σ4CB and intermediate ΣPAH concentrations in the sediment (Table 1), a low DNA integrity was established (0.4 < F < 0.5) (Figure 2e), which compares the F-value in specimens from stations 4, 8, and 9.

The data on the induction of the MO system in female seastars, measured as the hydroxylation of benzo[a]pyrene (BPH activity) are summarized in Figure 3d. In spite of the restricted number of samples analyzed, some evidence for the inductive effect of PCB congeners was found. At high Σ4CB concentrations (350–450 ng/g lipid) enhanced BPH activity was measured in pyloric caeca of seastars from three coastal stations (stations 10, 14, 17). However, one exceptionally high value for BPH activity was measured in seastars from the supposedly pristine reference site in the central North Sea (station 5). The percentage lipid extracted from the pyloric caeca of specimens from this station (5.4% ± 0.8) was significantly higher (p < 0.05; Student’s t-test) lower than the lipid contents in animals from all other stations (12), which varied from 8.2% ± 1.0 to 11.5% ± 1.2. It might be possible that lipid content affects BPH activities.

**Summary and Conclusions**

In summary, the data indicate that in dab and seastars of the southern and central part of the North Sea, specific molecular responses were measurable by means of biochemical techniques, sometimes in connection with unfavorable ambient circumstances (e.g., enhanced concentration levels of polycyclic (aromatic) hydrocarbons, such as PCBs or PAHs) or natural physiological changes within the organism (e.g., lipid content). However, of all causal relationships possible between the contaminants determined and responses in dab and seastars, only the correlation between the DNA integrity (F-value) in seastars and the sum of Σ4CB in seastar and Σ24PAH in sediment (Figure 4) revealed to be statistically significant (p < 0.001; linear regression; r = 0.767; n = 15). On the contrary, gradients in molecular responses (DNA damage, enzyme induction) could not be described in correspondence with a particular chemical gradient. Nevertheless, the organic microcontaminants considered in the present study, such as selected CB congeners and PAHs, do affect the physiologic status of organisms (2,9–13,17,26). However, under natural conditions, the magnitude of this impact is obscured by all types of environmental conditions and thus difficult to establish. Well-designed dose-response experiments often establish significant correlations and, even more important, they can help elucidate the toxic action of a chemical. On the contrary, it is very difficult to describe dose-response relationships in the field. On the basis of the present data the next example can be given. In the seastar, considerable differences in the DNA integrity were described, whereas the differences in BPH activity were small, with one exception (Figure 3d). Which environmental factors caused the observed molecular responses?

In a laboratory study, it was found that the nonortho planar CB126 and BaP induce the MO system (17). However, in the case of CB126 this occurred at levels 10 times higher than those found in the present study. The identification and quantification of CB126 still have to be confirmed by means of capillary two-dimensional gas-chromatography-ECD. Nevertheless, the attempt to relate the molecular responses measured in the specimens obtained from the different areas in the North Sea to CB126 indicated a negative relationship (p < 0.1; linear regression, r = 0.448, n = 15) with the F-value (Figure 5a). At increased CB126 concentrations, BPH activity increased significantly (p < 0.05; linear regression, r = 0.899, n = 5), if the data from station 5 were

![Figure 4](https://example.com/figure4.png)
excluded, because of the low lipid content of the pyloric caeca. (Figure 5b). PAH levels in the pyloric caeca were not measured in the present study, but in other field studies concentrations were found (27) in the range causing MO induction in laboratory studies (17). Not including station 5, the significant correlation (p<0.05; linear regression, r = 0.879, n = 5) between the BPH activity and the F-value (Figure 5c) revealed that the induction of the cytochrome P450-dependent MO system in Asterias rubens in catalyzing the BaP metabolism by means of hydroxylation of benzo[a]pyrene induces strand-breaks in the DNA. Apart from these considerations, attempting to understand possible causal relationships between environmental factors and the induced molecular responses, the ultimate impact of these responses on organisms or populations is still unclear. Likewise, it is not obvious yet how to project these responses into the model of homeostasis and compensation (4–6) or how to translate the responses into physiologic implications.

In conclusion, establishing the DNA integrity and the induction of the cytochrome P450-dependent monooxygenase system in dab and seastar elucidate the physiologic status of the individual organism and might be indicative of ambient impairment, but not necessarily related to anthropogenic or xenobiotic substances. Gradients in DNA damage and enzyme induction could not be described in correlation with chemical gradients along transects radiating into the North Sea. Significantly excess DNA strand breaks were found in liver and pyloric caeca of dab and seastar, respectively, obtained from different locations in the North Sea, but the sampling sites were not characterized by consistent environmental conditions (such as contamination with either PCBs or PAHs, low temperature).

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