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Evaluation of chromosomal damage by flow cytometry in turbot (*Scophthalmus maximus* L.) exposed to fuel oil

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Flatfishes, turbots (*Scophthalmus maximus*), were injected intraperitoneally with two doses of fuel oil number 2. Biliary metabolites were evaluated by fixed fluorescence to verify the efficiency of intoxication. Ethoxyresorufin-*O*-deethylase (EROD) activity was compared with chromosomal damage measured by flow cytometry. The analysis of biliary metabolites showed a good dose–response relation and constitutes a clear reference for the subsequent measurements. Comparing flow cytometry and EROD results, a shorter delay of response for EROD activity was obtained, but chromosomal damage was significant only after 1 week. The persistence of the EROD response was shorter, while the genotoxic signal still persisted after 1 month. The measurement of chromosomal damage allowed a good differentiation between the two tested doses. In the case of EROD activity, the results were less clear. The results suggest that within a few weeks after exposure to fuel oil number 2, the measurements of chromosomal damage by flow cytometry can be used to detect a dose-dependant genotoxic response in fish.

Keywords: fuel oil number 2, turbot, flow cytometry, EROD, bilary fluorescence, chromosomal damage.

Introduction

Fuels are widely prevalent pollutants in the marine environment, and hence polyaromatic hydrocarbons (PAH) are a group of xenobiotics of particular interest. PAH are present in fuels and have genotoxic properties (Krahn *et al*. 1986, Malins *et al*. 1987, Varanasi and Stein 1991, Vethaak *et al*. 1996, Stehr *et al*. 2003, Stentiford *et al*. 2003). Their effects on organisms are assessed classically by the use of biomarkers. This approach is a suitable strategy for studying sublethal effects of pollutants, providing early indication of possible adverse effects (Carajaville *et al*. 2000, Van der Oost *et al*. 2003).

Genotoxicity includes different types of genetic material modifications, e.g. DNA structure alteration (as DNA adducts, nucleotide base modification, points mutations, DNA strand breakage), chromosomal aberrations or variations in nuclear

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DNA content (Shugart 1999). In the present study, chromosomal damage was evaluated by the measurement of nuclear DNA content by flow cytometry (FCM). Chromosomal damage was chosen because, at a cellular level, it is an irreversible event resulting from both DNA damage and repair and effects last longer than other types of genetic damage such as single-strand breaks (Shugart 1999).

Flow cytometry assays have been developed to detect the changes in nuclear DNA content resulting from the chromosomal damage produced by clastogenic agents such as PAH (Easton *et al*. 1997, Easton 1999). Thus, FCM has been recently applied to the detection of the genotoxic effects of environmental pollutants to wildlife populations (Bickham *et al*. 1998, Custer *et al*. 2000, Marchand *et al*. 2003) and it provides a rapid test applicable to any tissue from which cellular or nuclear suspensions can be obtained. FCM is becoming a classic tool for the assessment of the impact of chronic pollution (Easton *et al*. 1997, Bickham *et al*. 1998, Easton 1999, Custer *et al*. 2000, Marchand *et al*. 2003), and for *in situ* bio-monitoring of irreversible genotoxic events in environmental risk assessment (Van der Oost *et al*. 2003).

The measurements of change in nuclear DNA content by FCM have not been evaluated to assess short-term effects in the context of accidental pollution. To check if this biomarker allows an efficient evaluation of short-term effects of clastogenic agents, the time-course of chromosomal damage was evaluated by FCM in turbot over 1 month after intoxication with fuel oil number 2.

The evaluation of the FCM measurement of chromosomal damage necessitates (1) a protocol of exposure to the xenobiotic allowing precise control of the intoxication doses; and (2) a comparison with biomarkers known to respond with a short delay.

Consequently, intoxications were performed by intra-peritoneal (i.p.) injection of different doses of fuel, a technique allowing one to obtain accurately different levels of intoxication. As biliary PAH metabolites are biomarkers of exposure (Krahn *et al*. 1984, 1986, Ariese *et al*. 1993, Lin *et al*. 1996), the efficiency of intoxication was checked by measuring PAH metabolites in bile by fixed fluorescence (FF). This method of screening PAH contamination in fish was described by Aas (2000) and Aas *et al*. (2000b).

PAH compounds are potent inducers of CYP1A in flatfish in laboratory experiments (Beyer *et al*. 1997, Van Schanke *et al*. 2001, Reynolds *et al*. 2003) as in field studies (Arinç *et al*. 2000, Miller *et al*. 2003). CYP1A induction, estimated by the measurement of EROD activity, has been widely used as a biomarker for planar organic contaminants such as PAH (Arinç *et al*. 2000, Whyte *et al*. 2000). As measurements of EROD activity allow early detection of PAH contamination in fish (1–8 days after intoxication) (Camus *et al*. 1998, Aas *et al*. 2000a), results obtained with FCM were compared with those obtained with EROD assays.

Materials and methods

Chemicals

Sucrose, dimethylsulfoxide (DMSO), dihydrate trisodium citrate, citric acid, resorufin, 7-ethoxyresorufin, bovine serum albumin (BSA), NADPH, EDTA, dithiotreitol (DTT), Hepes, and glycerol were purchased from Sigma (Saint Quentin Fallavier, France).

Fuel oil number 2, a heavy fuel oil similar to Erika oil containing approximately 22% saturate, 55% aromatics and 22% polar compounds, was supplied by the Centre de Documentation de Recherche et d'Expérimentations sur les pollutions accidentelles des eaux (Cedre, Brest, France). Corn oil (household) was used for the dissolution of fuel oil.

Fishes

Juvenile turbot, *Scophthalmus maximus* ($n = 205$; 347 ± 74 g; 28 ± 2 cm, mean $\pm$ SD), were purchased from a fish farm (France Turbot, 22 220 Trédarzec, France). They were acclimatized for 2 weeks in laboratory tanks. Each fish was labelled with a pit-tag. They were fed daily with dried pellets (aquaculture food Le Gouessant®, 4.5 mm diameter, total protein 54% of dry matter and crude fat 12% of dry matter) except during the 48 hours before sampling in order to avoid bile evacuation.

Experimental design

Experimental conditions. The fishes were maintained in three fishery tanks (1200 litres). Water flow was regulated in each tank at 540 l h^{-1}. Filtration and aeration were ensured by a water circulation system through two tanks (stocking tank of 12 000 litres and an alimentation tank of 1000 litres) and two filters (physical and biological) (figure 1).

Experiments were carried out in November and December 2002. The light regime was set according to the season: 14 h light, 10 h dark. Water salinity (35–36‰), water pH (7.97–8.25), oxygen concentration (228 ± 18 µmol l^{-1}) and sea water temperature ($15^{\circ}C \pm 2$) were measured daily.

Contamination, sampling schedule and sample preparation. To evaluate the effects of two controlled doses of fuel oil, fishes were divided into four groups: control, sham (corn oil injection of 0.2 ml kg^{-1}), group '50' and group '150'. Groups 50 and 150 had, respectively, doses of 50 and 150 mg kg^{-1} fuel diluted in corn oil by injections of 0.2 ml kg^{-1}.

Eight to ten fishes were then sampled in each group 1, 3, 8, 14 and 30 days after contamination. Fish were identified and samples of 500 µl blood were taken from the caudal vein in a syringe containing 500 µl freezing solution (sucrose: 250 mM; trisodium citrate: 40 mM; 5% DMSO) (Vindeløv and Christensen 1990). Blood samples were kept in ice for 10 min, frozen in liquid nitrogen and stored at $-80^{\circ}C$. The fishes were killed by a sharp blow to the head. The gallbladder and liver were excised, weighed, washed in KCl solution (0.15 M), frozen in liquid nitrogen and stored at $-80^{\circ}C$ for later analyses.

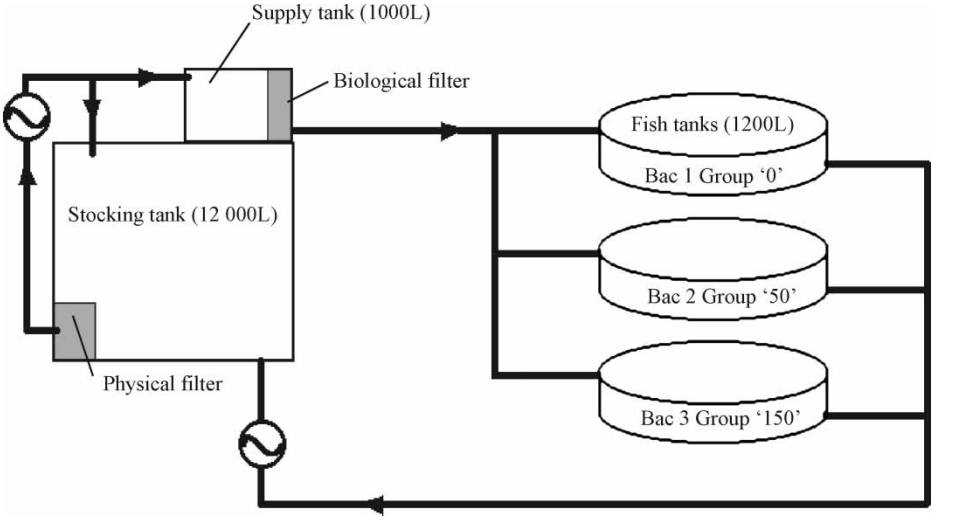

Figure 1. Stabulation system. The sea water pumped from the stocking tank (12 000 litres) was filtered by a physical filter. It was then sent to the supply tank or returned to the stocking tank to oxygenate the water. From the supply tank and after biological filtering, sea water was injected in the three fish tanks at the same flow rate (540 l h^{-1}).

Analyses

PAH measurements. One, 7, 14 and 28 days after injection, samples of seawater were taken to verify that the water in the three exposure tanks was not contaminated by oil. A volume of 1 litre was collected in heated (500°C) Duran glass bottles. Samples were extracted with dichloromethane pestipur quality. After separation of the organic and aqueous phases, water was extracted two additional times by the same volume of dichloromethane (2 × 30 ml). The combined extracts were dried by filtering through anhydrous sodium sulfate (pesticide grade) and concentrated to 1 ml by means of a Turbo Vap 500 concentrator (Zyman, Hopkinton, MA, USA). PAH detection was performed by gas chromatography/mass spectroscopy (GC/MS) essentially following published procedures (Douglas *et al*. 1992). Separation was performed on a Hewlett Packard HP 5890 gas chromatograph fitted with a 30 m × 0.25 mm fused silica capillary column with 5% cross-linked phenyl methyl silicone as the stationary phase. Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. Samples of 1 µl were injected automatically by an HP 6890 injector. The column temperature was set to 45°C for the first 4 min, increased 8°C min^{-1} to 270°C then increased 5°C min^{-1} to 310°C and maintained at 310°C for 5 min. Mass spectral data were obtained with an HP 5972 mass selective detector at an energy of 70 eV over a mass range of 35–500 atomic mass units in total-ion mode.

The characterization of the original oil was performed according to the same method.

Spectrofluorimetric measurements. Fluorescence analyses of bile samples were performed with a Kontron SFM 25 (Aas *et al*. 2000b).

Synchronous fluorescence spectrum (SFS) was used to determine the maximum fluorescence in samples. The scan range was 230–500 nm for excitation and 272–542 nm for emission, with sweeping speed of 80 nm min^{-1} and a 40 nm difference between the excitation and emission wavelengths (Aas *et al*. 2000b). The peak observed at the couple 343/383 nm was characteristic of 4-ring PAH compounds (pyrene metabolites) (Krahn *et al*. 1984, 1986, Lin *et al*. 1996, Aas 2000). Consequently, this value was used to determine the fluorescence intensity of bile samples by a fixed fluorescence (FF) method.

EROD analyses. EROD activity was measured with the method modified from Burke and Mayer (1974) and Monod *et al*. (1994). Microsomal protein was determined by Lowry's (1951) assay using BSA as a standard.

Liver samples were homogenized in 4 vols ice-cold buffer (pH 7.6, 25 mM Hepes, 0.25 mM EDTA, 10% v/v glycerol, 1 mM DTT). The homogenates were centrifuged (20 min, 10 000*g*); then the supernatants were ultracentrifuged (60 min, 100 000*g*). Pellets were suspended in phosphate buffer (50 mM, pH 7.4, 20% v/v glycerol, 1 mM EDTA, 1 mM DTT). The reaction mixture was composed of 400 µl phosphate buffer (200 mM, pH 7.6), 100 µl microsomes and 8 µl NADPH (100 µM). The reaction was started with the addition of 8 µl ethoxyresorufin (0.5 mM) and incubated 20 min at 25°C; the reaction was stopped by addition of 2 ml acetone.

EROD activity was measured with a spectrofluorimeter Jasco FP-6200 with resorufin as standard (range of concentration 5–125 µM) at 537/583 nm and was expressed as picomoles resorufin produced min^{-1} mg^{-1} microsomal protein.

Flow cytometry. Blood samples half-diluted in citrate buffer were thawed in ice and stained with propidium iodide at least 30 min before analysis (Vindeløv and Christensen 1990). To control the variations in flow-cytometry responses, chicken blood was used as an internal standard and added to each blood sample. DNA profiles from 20 000 stained erythrocyte nuclei were generated on a Becton Dickinson FACSCalibur flow cytometer. Half-peak coefficient of variation (CV) of blood cell DNA content was determined for each profile with Win MDI 2.8 software. Samples with abnormal peaks (multiple peaks or asymmetries) were discarded and the internal standard CV stability checked. The fish red blood cell CVs were corrected for instrument drift by subtraction of the chicken CVs, the resulting difference being the parameter analysed (Easton *et al*. 1997).

Statistical analyses

Statistical tests in flow cytometry, biliary fluorescence and EROD assays were performed with Statistica (Version 6.0, Statsoft). Normality was checked using Lilliefors test; variance homoscedasticity was evaluated using a Bartlett test. Flow cytometry results were gaussian and expressed as mean ± SEM; a two-way ANOVA was performed for analysis of chromosomal damage. Results of biliary fluorescence and EROD activity were not gaussian and were expressed as median ± EQ. Before statistical analysis, those results were normalized (with a square-root transformation) and an ANOVA II test performed. In the case of EROD results, on the 30th day the group 150, j30 had only three points ($n = 3$). This result could not be analysed statistically. Hence, the ANOVA II on EROD results could only be performed between days 1 and 14.

Statistical analysis of fixed fluorescence, EROD assay and flow cytometry results showed there was no significant difference between the control and the sham fish group, so these two groups were pooled and named group '0'.

Results

The mortality rates over the whole experiment period were 0, 1 and 3.4% for groups 0, 50 and 150, respectively.

PAH measurements

Table 1 displays the PAH analysed in the fuel oil used for the intoxications.

During the experiment, the same PAHs were analysed in water at different times. Results are given in table 2. Most PAH concentrations were under the detection threshold. On day 1, some PAHs were detected, but their concentrations greatly decreased from day 7 after intoxication.

Fixed fluorescence

The fluorescence signal (expressed in figure 2 in relative fluorescence unit, or RFU) was subjected to a square-root transformation before statistical analysis. Dose (0, 50, 150 mg kg^{-1}) and time effects were analysed with the ANOVA II test: there were significant time effects ($p=0.000$, $F=15.5$, $v=4$), dose effects ($p=0.000$, $F=276.0$, $v=2$) and a link between the two parameters ($p=0.000$, $F=11.6$, $v=8$).

The fluorescence signal was higher in the exposed groups when compared with group 0 whatever the considered sampling days.

In group 50, the RFU level was maximal 1 week after injection (day 8, RFU = 28.1 ± 20.3) and then decreased from days 8 to 30, but it remained higher than in group 0. In group 150, the RFU median increased during the first 14 days to 62.9 ± 33 RFU, but was lower at day 30 (32.0 ± 35.1 RFU).

Table 1. Analysis of 16 priority PAH of the United States Environmental Protection Agency (US EPA) list in fuel oil. PAH detection was performed by gas chromatography/mass spectroscopy (GC/MS).

Naphtalene	547.5 ± 71.2
Acenaphthylene	36.1 ± 2.2
Acenaphthene	149.5 ± 7.5
Fluorene	200.4 ± 10.0
Phenanthrene	1075.9 ± 64.6
Anthracene	228.6 ± 29.7
Fluoranthene	81.2 ± 8.9
Pyrene	603.2 ± 66.4
Benz[a]anthacene	347.4 ± 20.8
Chrysene	604.9 ± 18.2
Benzo[b+k]fluoranthene	97.4 ± 12.7
Benzo[a]pyrene	194.9 ± 7.8
Dibenz[a,h]anthracene	70.6 ± 12.7
Benzo[ghi]perylene	77.1 ± 4.6
Indeno [1,2,3-cd] pyrene	28.6 ± 5.4

Results in $\mu g\ g^{-1}$ hydrocarbons ($n=3$, means $\pm$SD).

Table 2. Analysis of PAH in the water circulation system during the experiment.

	Day 1	Day 7	Day 14	Day 28
Naphtalene	69.280 ± 9.610	0.766 ± 0.426	2.150 ± 1.737	2.000 ± 3.090
Acenaphthylene	n.d.	n.d.	n.d.	n.d.
Acenaphthene	n.d.	n.d.	n.d.	n.d.
Fluorene	n.d.	n.d.	n.d.	n.d.
Phenanthrene	3.780 ± 0.301	0.198 ± 0.337	0.573 ± 0.827	0.435 ± 0.468
Anthracene	0.118 ± 0.050	0.007 ± 0.012	0.015 ± 0.001	0.021 ± 0.024
Fluoranthene	0.335 ± 0.026	0.037 ± 0.059	0.105 ± 0.131	0.043 ± 0.170
Pyrene	0.974 ± 0.491	0.073 ± 0.150	0.257 ± 0.328	0.065 ± 0.076
Benz[a]anthacene	n.d.	n.d.	n.d.	n.d.
Chrysene	n.d.	n.d.	n.d.	n.d.
Benzo[b]fluoranthene	n.d.	n.d.	n.d.	n.d.
Benzo[k]fluoranthene	n.d.	n.d.	n.d.	n.d.
Benzo[a]pyrene	n.d.	n.d.	n.d.	n.d.
Dibenz[a,h]anthracene	n.d.	n.d.	n.d.	n.d.
Benzo[ghi]perylene	n.d.	n.d.	n.d.	n.d.
Indeno [1,2,3-cd] pyrene	n.d.	n.d.	n.d.	n.d.

Results are ng l^{-1} sea water (each point is the mean ± SD of three measurements performed in the three tanks).

n.d., Not detected.

EROD assay

EROD activity (expressed in figure 3 in pmol min^{-1} mg^{-1} protein) was subjected to a square-root transformation before statistical analysis. Dose (0, 50, 150 mg kg^{-1}) and time effects (days 1, 2, 8, 14) were analysed with the ANOVA II test: there were significant time effects ($p = 0.000$, $F = 12.30$, $v = 3$), dose effects

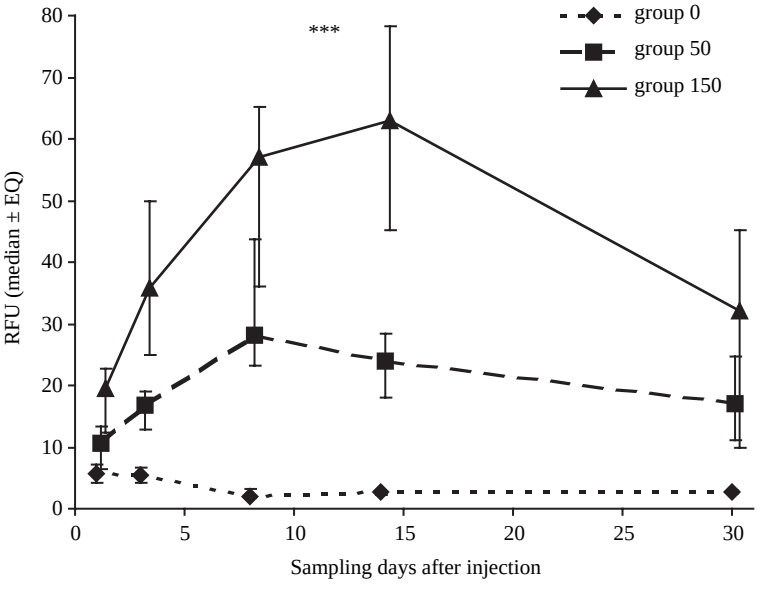

Figure 2. Time–response of 1-OH-pyrene metabolites levels (median ± EQ) in the bile of turbot after i.p. injection of 0, 50 and 150 mg kg^{-1} fuel oil number 2. Levels are expressed as relative fluorescence unit (RFU), $n = 6–10$ per group. A clear dose–response is observed. Significance level: ***$p < 0.001$.

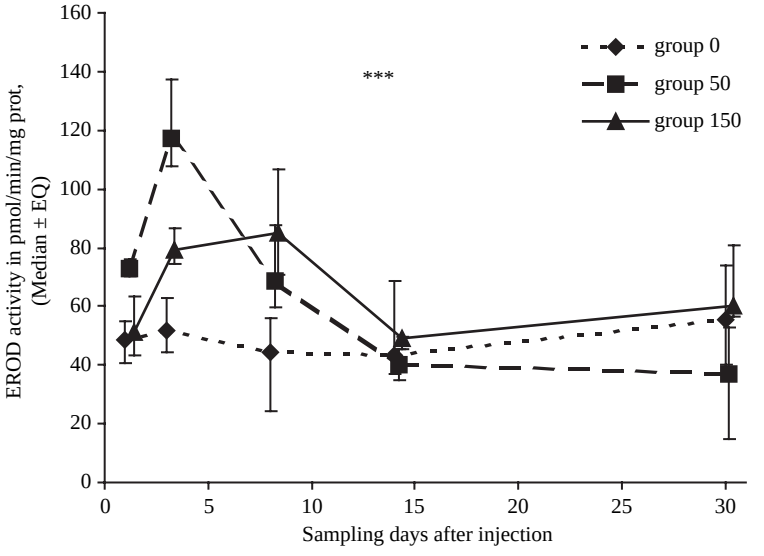

Figure 3. Hepatic EROD activity (median ± EQ, pmol min^{-1} mg^{-1} protein) in turbot after i.p. injection of 50 and 150 mg kg^{-1} fuel oil number 2, $n = 6–10$ excepted in the group 150, j30 where $n = 3$; the 30th day has been removed from statistical analysis. A dose–response was observed. Significance level: ***$p < 0.001$.

($p = 0.000$, $F = 16.84$, $v = 2$) and a link between the two parameters ($p = 0.000$, $F = 5.88$, $v = 6$).

In group 0, EROD activity was constant during the whole experimentation.

In group 50, EROD values display a large and early increase: 1.5-fold the value of group 0 at day 1 and 2.3-fold at day 3. Then it rapidly decreased to the control value at day 14.

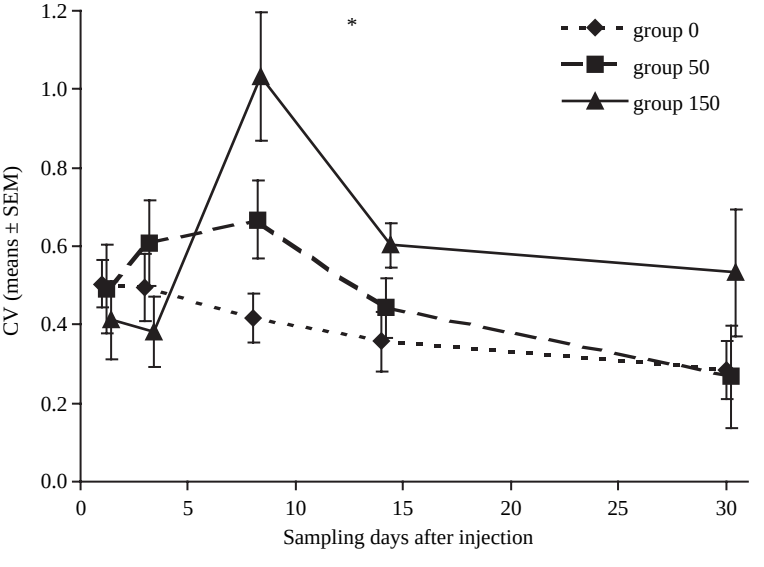

Figure 4. CV dispersion of DNA contents of turbot erythrocytes (means ± SEM) after i.p. injection of 50 and 150 mg kg^{-1} fuel oil number 2. DNA damage is expressed as a variation of CV, $n = 7–10$ per group. A dose–response was observed. Significance level: *$p < 0.05$.

In group 150, the EROD activity moderately increased during the first 8 days. Maximal EROD activity for this intoxication dose was 1.70-fold the value of group 0 day 8. At day 14, the values were similar in groups 150 and 0.

Flow cytometry

Dose (0, 50, 150 mg kg^{-1}) and time effects were analysed with the ANOVA II test: there were significant time effects ($p = 0.002$, $F = 4.440$, $v = 4$), dose effects ($p = 0.023$, $F = 3.877$, $v = 2$) and a link between the two parameters ($p = 0.003$, $F = 3.148$, $v = 8$).

The CVs of group 0 decreased slowly between days 1 (0.50 ± 0.06) and 30 after injection (0.29 ± 0.08). The CVs of group 50 increased to day 8 (1.6-fold the value of control group), then decreased to the CV group 0 value at day 30. The group 150 CV increased sharply at day 8 (2.5-fold the control value), then decreased to day 30, but remained higher than the mean CV of group 0 (1.7-fold the value of group 0 at day 14 and 1.9-fold at day 30).

Discussion

The aim was to test the potential interest of chromosomal damage measured by FCM as a biomarker for accidental fuel pollution. Hence, the delay and duration of response were evaluated after i.p. injection of fuel oil in turbot. During the experiment, exposure to pollutant was evaluated with biliary metabolites and FCM results were compared with EROD measurements, a classical biomarker.

After intoxication, once into the organism, the constituents of fuel oil can be metabolized by phase I enzymes (CYP1A). In fishes, this pathway of detoxification is very active and there is generally no bioaccumulation. The metabolites of PAH are then excreted into the bile, and one of the major metabolites observed after fuel contamination is 1-hydroxy-pyrene. The modifications of CYP1A and biliary metabolites are very rapid after an intoxication by fuel oil, so although they are biomarkers of effect they are used to indicate exposure. Moreover, the PAH and their metabolites may bind covalently or cause damage to cellular macromolecules (DNA or RNA). If this damage is not repaired, it will be lasting in daughter cell lines through mitosis. Damaged daughter cells can have different DNA contents, and this abnormality can be detected with flow cytometry. However, modifications of nuclear DNA content are considered to be long to appear, with delays of weeks to months (Shugart 1999).

Results show that some PAH were detected in the sea water at the different sampling times. However, the concentrations decreased during time and were always very low, except just after oil injection (day 1). Only aromatic compounds with fewer rings (not more than three) were detected. This could be explained by the fact that they are more soluble than those with more rings (Yaws *et al*. 1993).

The fluorescence of biliary metabolites is a biomarker allowing evaluation of fuel oil exposure (Lin *et al*. 1996, Camus *et al*. 1998, Aas *et al*. 2000b). In the present study, this technique was used to obtain an evaluation of the level of intoxication and the kinetics of PAH elimination.

The results showed a dose–response relation was in good agreement with what was reported by Aas *et al*. (1998) and Van Schanke *et al*. (2001): high doses (150 mg kg^{-1}) induced higher and more durable effects (60 RFU, maximal response after 2 weeks) than lower doses (50 mg kg^{-1}, 28 RFU, maximum at day 8).

Evaluation of the fluorescence of biliary metabolites constitutes a basis clear enough for subsequent measurements: EROD activity and the evaluation of chromosomal damage.

The results of EROD activity in liver appear less reliable than bile fluorescence as EROD measurements are highly variable as was observed in other studies (Whyte *et al*. 2000, Telli-Karakoç *et al*. 2002).

A total of 50 mg kg^{-1} i.p. injection of fuel was followed by a rapid response of hepatic cytochrome P4501A activity estimated from EROD measurement. Recovery of the basal level was completed after 14 days.

When fishes were intoxicated with a 150 mg kg^{-1} dose of fuel, the response was lower and delayed. This observation could be the consequence of a partial inhibition of synthesis and/or activity of cytochrome P4501A by a high dose of contaminants (Whyte *et al*. 2000).

At day 30, only three samples could be obtained and analysed for group '150'. So, the results obtained at this time were not as reliable as the others and were not included in the statistical analysis. However, the fact that at day 14, EROD activity was no longer significantly different from control values shows that the duration of the response was of the same order than when fishes where intoxicated with a 50 mg kg^{-1} dose.

Genotoxic effects of fuel oil were estimated from an enlargement of profiles corresponding to the DNA quantity measured in red blood cell nuclei.

Clastogenic effects of fuel oil and PAH have been observed in field studies and in the case of long-term exposition to pollutants, e.g. in sea-otter exposed to Exxon Valdez pollution (Bickham *et al*. 1998), and in ducks (lesser scaup) wintering on heavily polluted zones (Custer *et al*. 2000).

In the present experiment, the mean CV was increased at day 8 and this response was dose dependant as it was much higher at 150 than at 50 mg kg^{-1}. DNA abnormalities disappeared 2 weeks after i.p. injection in the case of the lowest dose of intoxication, whereas they remained significantly different from control up to day 30 for the highest fuel dose.

The measurement of chromosomal damage by FCM is now a classical biomarker allowing the evaluation of the long-term impact of chronic pollution (Easton *et al*. 1997, Lowcock *et al*. 1997, O'Connor *et al*. 2001). Comparing FCM and EROD results after an i.p. intoxication with fuel oil, a shorter delay of response for EROD activity compared with the genotoxic response (CV) was obtained. EROD induction was observed from the first day after intoxication, while genotoxic responses were clearly detected after 1 week.

The persistence of the effect on EROD activity was shorter: 14 days after intoxication there was no more difference between the different groups. To the contrary, in the case of the higher dose of intoxication, the genotoxic signal still persisted after 1 month.

The measurement of chromosomal damage by flow cytometry allowed a good differentiation between the impact of the two tested doses: from the eighth day, the response is dose dependant, and the higher dose of intoxication remains higher than the weak dose until day 30. In the case of EROD activity, the results are less clear: the response is higher for the weak dose of fuel during the first 3 days, and the two doses could not be separated at day 8.

Finally, the dispersion of results was less variable for CV than for EROD activity and the response range compared with dispersion was more important for CV than for EROD.

The results in fishes suggest that within a few weeks after an accidental pollution by hydrocarbons, the measurement of chromosomal damage by flow cytometry could be a fast and easy tool to detect a dose-dependant genotoxic response.

In this study, animals were exposed to fuel oil by i.p. injection. This method allows precise control of the intoxication dose but is very different from conditions of intoxication arising in the case of an accidental release of fuel at sea: the fuel doses administered to the fishes are high and i.p. injection is not representative of natural uptake mechanisms. Hence, this work will be followed by an evaluation of chromosomal damage by FCM with fishes exposed to the dissolved fraction of fuel oil in sea water.

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