QUALITATIVE ASSESSMENT OF GENOTOXICITY USING RANDOM AMPLIFIED POLYMORPHIC DNA: COMPARISON OF GENOMIC TEMPLATE STABILITY WITH KEY FITNESS PARAMETERS IN DAPHNIA MAGNA EXPOSED TO BENZO[a]PYRENE

FRANCK A. ATIENZAR,* MERCEDES CONRADI, ANDREW J. EVENDEN, AWADHESH N. JHA, and MICHAEL H. DEPLEDGE

Plymouth Environmental Research Center (PERC) and Department of Biological Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, United Kingdom

(Received 16 September 1998; Accepted 1 February 1999)

Abstract—A method of DNA profiling using the random amplified polymorphic DNA (RAPD) was used to assess toxicant-induced DNA effects in laboratory populations of Daphnia magna exposed to varying concentrations of the genotoxic hydrocarbon benz[a]pyrene. These effects, represented by changes in the RAPD profiles, were compared with a number of key ecological fitness parameters (age-specific survival, age-specific fecundity, net reproductive rate, and intrinsic rate of population increase). Not only was the RAPD profiling method shown to be a rapid and reproducible assay of toxicant-induced DNA effects, but the qualitative measure of genomic template stability compared favorably with the traditional indices of fitness. The RAPD profiles, however, exhibited higher sensitivity in detecting toxic effects. The significance of these findings for future ecotoxicological studies is discussed.

Keywords—Random amplified polymorphic DNA profiles  Benzo[a]pyrene  Daphnia magna  Genomic template stability  Fitness parameters

INTRODUCTION

Pollutants with genotoxic potential are of great concern to many ecotoxicologists. Once released, these agents have the capability not only to cause morbidity and/or mortality in the exposed organisms but potentially may induce higher order changes such as alterations to population dynamics and changes to biological diversity at both intra- and interspecies levels [1–3]. Such changes may initiate direct and catastrophic ecological consequences. The genotoxicity of pollutants is directly related to their effects on the structure and function of DNA molecules, which may be determined using a number of laboratory methods (e.g., [4,5]). However, while such pollutant–DNA interactions can be readily demonstrated using such methodology, there have been few direct experimental demonstrations of the wider relationships between DNA effects and their subsequent consequences at higher levels of biological organization [6,7]. To address this problem, it is necessary to develop reliable and reproducible genotoxicity assays that may then be used in conjunction with traditional assays detecting any impairment of classical Darwinian fitness parameters (e.g., growth, reproductive output, viability of offspring).

In the field of genotoxicity, advances in molecular biology have led to the development of a number of selective and sensitive assays for DNA analysis. The random amplified polymorphic DNA (RAPD) and the arbitrarily primed polymerase chain reaction (AP-PCR) techniques are two semiquantitative methods that have been used in genetic mapping, taxonomy, and phylogeny [8–10] and for the detection of various kinds of DNA damage and mutations [11–16]. Although RAPD and AP-PCR are very similar techniques (the methods have been often described as the same procedure, e.g., [15,17,18]), Meunier and Grimont [19] have defined procedural differences between the techniques. The detection of AP-PCR amplified product is facilitated by incorporation of α-32P-dATP during the last 10 cycles of the polymerase chain reaction (PCR) before electrophoresis is carried out in acrylamide gel prior to visualization by autoradiography. In contrast, RAPD fragments are detected after agarose gel electrophoresis and ethidium bromide staining. Therefore, to avoid any confusion, the definition described by Meunier and Grimont [19] has been adopted in this paper. Both techniques are based on the selective amplification of genomic sequences that, by chance, are flanked by adequate matches to an arbitrarily chosen oligonucleotide primer sequence. If two template genomic DNA sequences are different, the PCR products will display different banding patterns or profiles when these products are subjected to electrophoresis [9,20]. Detection of genotoxic effect using these techniques involves the comparison of profiles generated from control (unexposed) and treated (exposed) DNA.

The aims of this study were to further evaluate the potential of the RAPD technique to detect DNA damage as an ecotoxicological tool and to compare changes in RAPD profiles induced by the genotoxic polycyclic aromatic hydrocarbon, benz[a]pyrene (BaP) with fitness parameters measured in a laboratory population of the cladoceran Daphnia magna.

MATERIALS AND METHODS

Culture of Daphnia magna

Daphnia magna (D. magna, clone 5) were provided by Zeneca’s environmental laboratory at Brixham, United Kingdom, and were cultured in our laboratory. The animals were maintained in Elendt’s medium [21] at a temperature of 20°C (±1°C) with a photoperiod of 16 h light (1,000 lux):8 h dark. Populations of 40 individuals were maintained in 1 L of me-
dium contained in 2-L tall-form glass beakers (Sigma, Poole, UK). The medium was changed three times per week. Animals were fed the algae *Chlorella vulgaris* (1.2–2.4 × 10^7 cells/daphnid/d) and a booster solution of Frippack microencapsulated food (Salt Lake Brine Shrimp, Grantsville, UT, USA). This extra source of carbon was incorporated into the culture medium at a ratio of 0.023 mg Frippack for every 1.2 × 10^7 algal cells.

**Preparation of test solutions**

Test solutions for chronic exposures were prepared from stock solutions of BaP at a concentration of 2 mg/ml in dimethylformamide (DMF). Once prepared, test solutions were stored at 4°C prior to use. Both BaP solutions and the DMF solvent controls were added to the *Daphnia* culture media in a volume of 100 μl of DMF in 1 L of medium (ensuring a level below the 0.05% maximum percentage of the solvent recommended by the U.S. Environmental Protection Agency) [22].

**Toxicity tests**

**Acute toxicity.** The acute toxicity of BaP was assessed by determining the LC50 of the chemical for *D. magna* over a period of 48 h. Freshly born neonates (less than 48 h) were exposed in replicate groups of 20 to concentrations of BaP equivalent to 0.1, 0.15, 0.2, 0.25, and 0.5 mg/L. Animals were fed (see culture of the organism) during the test, and surviving animals were counted to determine the 48-h LC50.

**Chronic toxicity.** The chronic toxicity test of BaP to *D. magna* was performed under the same experimental conditions as for the acute toxicity tests, with a blank control, DMF solvent control, and BaP concentrations of 0.0125, 0.025, 0.05, 0.1, and 0.2 mg/L, respectively. It is noteworthy that BaP concentrations used in the chronic test were lower than the 48-h LC50 determined in the acute toxicity (see the Results section). Surviving animals were counted at 0, 3, 5, 7, 10, and 14 d. Moribund, nonswimming animals were removed from culture at regular intervals on and between counting days. These animals were placed individually in 1.5-ml microcentrifuge tubes, snap frozen in liquid nitrogen, and stored at −80°C prior to DNA extraction and RAPD profiling.

**Growth and reproductive measurements**

The length of *D. magna* (apex to base) surviving at day 0, 3, 5, 7, 10, and 14 d was measured by video capture and image analysis using a Quantimet 570 image analyzer (Cambridge Instruments, Cambridge, UK). Neonates were counted and recovered at daily intervals. Once pooled, each batch of neonates was snap frozen in liquid nitrogen and stored at −80°C prior to DNA extraction and RAPD.

**Calculation of fitness parameters**

The intrinsic rate of natural increase of the *D. magna* population, *r* _w_, was calculated using Lotka’s equation, \( \Sigma l_m e^{-\alpha t} = 1 \) [23,24]. For a cohort of animals observed from birth to death at regular intervals, *x* is the age in days, *l* _x_ is the age-specific survival (number of living females on day *x*/number of females at start of life table), and *m* _x_ is the age-specific fecundity (number of new-born individuals produced on day *x*/number of living females on day *x*). Realized fecundity (*U* _x_ ) was also calculated for the test populations (*U* _x_ = *l* _x_ *m* _x_). Using this data, the net reproductive rate (*R* _n_ ) can also be calculated, as *R* _n_ represents *U* _x_ summarized over the entire test period (\( R_n = \Sigma l_m = \Sigma U_x \)). Minimum generation time (\( T_{min} \)) was calculated by measuring the time that elapsed between birth and the deposition of the first batch of offspring. The interbrood time (*Bt*) was measured as the time (in days) between clusters or broods.

**Extraction of DNA from Daphnia magna**

Total DNA from *D. magna* was extracted and purified using either a conventional phenol/chloroform method or a commercially available extraction kit (IGi Genie DNA extraction Kit, Immunogen International, Sunderland, UK). Two methods were used because traces of organic compounds may inhibit PCR reactions, and one of the methods could have failed to produce DNA of sufficient quality to obtain robust RAPD profiles. Furthermore, both protocols of DNA extraction proved to generate similar RAPD patterns (data not shown). The conventional DNA extraction procedure involved the homogenization of single *D. magna* in 400 μl of sperm lysis buffer (100 mM Tris-HCl, pH 8; 500 mM NaCl; 10 mM ethylendiaminetetraacetic acid [EDTA], pH 8; 1% SDS; 2% mercaptoethanol) followed by RNase treatment (40 μg, 37°C for 1.5 h). The DNA was then extracted in phenol (pH 8) and chloroform/isooamyl alcohol (1:1). The DNA was finally precipitated by two volumes of ice-cold ethanol in the presence of 3 M sodium acetate (1/10 of the DNA volume) and was incubated at −80°C overnight. Precipitated DNA was harvested by centrifugation, dried in air, and the final pellet dissolved in sterile analytic grade water.

In the second procedure, individual *D. magna* were homogenized in 100 μl of sperm lysis buffer and treated with RNase (10 μg, 37°C for 1.5 h). The DNA extraction was performed using the protocol supplied by the manufacturer (DNA binding silica resin, IGi, Sunderland).

**Generation of Daphnia magna DNA profiles using RAPD**

The DNA profiles of *D. magna* were generated in RAPD reactions performed in a reaction volume of 25 μl as described previously [18]. The decamer oligonucleotides, OPA9 (GGTGAACGCC) and OPB7 (GGTGACGCAG), were obtained from Operon Technologies (Southampton, United Kingdom). Approximately 20 ng of *D. magna* genomic DNA was subjected to RAPD amplification with a primer concentration of 2 μM, a deoxy-trinucleotide phosphate (dNTP) concentration of 0.33 mM, and a MgCl₂ concentration of 5.11 mM in the presence of 2.8 units of *Taq* DNA polymerase and 1X reaction buffer (10 mM Tris-Cl, pH 8.3, 50 mM KCl, 0.1% Triton-X 100, 0.01% gelatin, 2.5 μg bovine serum albumin). All PCR chemicals were obtained from Immunogen International (Sunderland) except when otherwise mentioned. Thermal cycling parameters consisted of 5-min denaturation (95°C) followed by 40 cycles of 1-min denaturation (95°C), 1-min annealing at 50°C, and 1-min extension [74°C, with the final extension period adjusted to 10 min]. Reaction mixtures were stored at 4°C prior to use.

**Agarose gel electrophoresis and analysis of DNA profiles**

Electrophoresis of RAPD reaction products was performed in 1.2% w/v agarose, using a Tris-borate-EDTA buffer (TBE) system (1X TBE = 90 mM Tris-base, 90 mM boric acid, and 2 mM EDTA). Amplified DNA was mixed with one-fifth volume of gel loading buffer (analytical grade water containing 25% ficoll, 0.25% bromophenol blue, and 0.25% xylene cy-
anal), and 15 μl of this solution was loaded onto the agarose gel. Chemicals used were from Sigma (Poole, UK) unless otherwise mentioned. For comparison, DNA molecular size marker (1 Kb marker; Gibco BRL [Paisley, UK]) was used for each agarose gel. In all gels, the bands visualized were, from top to bottom, 3,054, 2,036, 1,636, 1,018, 517, 506, 396, 344, and 298 bp. The DNA samples were subjected to electrophoresis at 80 volts for 7 h, after which the gels were stained in a 1× TBE solution containing ethidium bromide (0.015% v/v) for a period not less than 40 min. Gels were photographed under ultraviolet illumination using a Polaroid camera (CU-5) (Cambridge, MA, USA). Images of each gel were also captured using a Kodak DC40 digital camera (Eastman Kodak, New York, NY, USA) and the DNA profiles analyzed using Kodak Digital Science® 1 D Image Analysis Software (Eastman Kodak).

**Estimation of genomic template stability and transformation of the data**

Each separate DNA effect observed in RAPD profiles (disappearance of bands, appearance of new bands, and variation in band intensities in comparison to control profiles) was given a score of +1 and the average calculated for each experimental group of animals with primers OPA9 and OPB7. The template genomic stability (percent) was calculated as 100 – (100/n)a, where n is the number of bands detected in control DNA profiles and a is the average number of changes in DNA profiles. To compare the sensitivity of each parameter (genomic template stability, lx, mx, rmx, and Rm), changes in these values were calculated as a percentage of their control value (set to 100%).

**Statistical analyses**

The LC50 value, confidence limits, and Chi-square goodness of fit were determined according to the method of Finney [25]. Differences among growth rates were calculated using multiple regression analysis as in Conradi and Depledge [26]. Briefly, the data were log transformed to homogenize the variances. Because the number of surviving animals decreased as the toxic effect increased, the regressions were weighted with n½ (where n is the number of the surviving animals). Correlation and analysis of variance (ANOVA) were performed using the computer software package Statgraphics (Statgraphics plus for Windows, version 2.1, Statistical Graphics, Princeton, NJ, USA). Changes in genomic stability and key fitness parameters (age-specific survival lx, age-specific fecundity mx, net reproductive rate Rm, and intrinsic rate of population increase Rm) were also statistically tested using ANOVA (Statgraphics). The least significant differences (LSD) test was used to reveal statistical differences.

**RESULTS**

**Acute toxicity**

Using survivorship data, the 48-h LC50 of BaP for D. magna (clone 5) was 0.25 ± 0.04 mg/L BaP (χ2 = 63.6, p < 0.005).

**Chronic toxicity**

Demographic trends for the populations of D. magna exposed to differing concentrations of BaP are shown in Table 1. From these results, it can be seen that, with increases in toxicant concentration in the range 0.025 to 0.2 mg/L BaP,
Fig. 1. Variation in (a) number of living *Daphnia magna* per replicate, (b) growth, and (c) total number of offspring in populations of *D. magna* exposed to varying concentrations of BaP, including 0 (control); 0 (solvent control); 0.0125; 0.025; +, 0.05; ▲, 0.1; and ●, 0.2 mg/L BaP. ** indicates a significant difference from control (p < 0.01).

Changes in fitness parameters

Alterations to the key fitness parameters, i.e., age-specific survival (l), age-specific fecundity (m), net reproductive rate (R), and intrinsic rate of population increase (r), and intrinsic rate of population increase (r), are presented in Table 1 and Figure 3, before and after transformation, respectively. The *D. magna* exposed to concentrations higher than 0.05 mg/L exhibited reduced life spans compared to the controls. Age-specific fecundity in blank and DMF controls was maximal on day 8, while animals exposed to both 0.0125 and 0.025 mg/L presented their maximal values on day 10 (data not shown). The intrinsic rate of natural increase and the net productive rate were also shown to be significantly reduced by increasing BaP concentrations above 0.0125 mg/L (p < 0.001). R and m appear to be the most sensitive fitness parameters, as r was calculated to be only significantly different at 0.1 and 0.2 mg/L BaP. None of the fitness parameters appeared to be altered at the lowest hydrocarbon concentrations.

The RAPD DNA profiling

The DNA amplified was extracted from moribund, non-swimming (but alive) organisms after 14, 7, and 5 d at 0.05, 0.1, and 0.2 mg/L BaP, respectively. For the other groups (i.e., control, control + DMF, 0.0125, and 0.025 mg/L BaP), organisms were sacrificed after 14 d. In total, 10 oligonucleotide primers were used to analyze the results. However, depending on the sequence of the primers, changes in RAPD profiles obtained from the exposed population occurred or not. The DNA profiles generated by two of them (primers OPA9 and OPB7) are shown in Figure 2. The patterns show significant differences between unexposed and exposed individuals, with visible changes in the number and size of amplified DNA fragments and both increases and decreases of DNA band intensities. Extra bands appeared with both primers OPA9 (two new PCR amplification products labeled 9-1 and 9-2; Fig. 2A) and OPB7 (three new main bands labeled 7-1, 7-2, and 7-3; Fig. 2B). The reproducibility of the RAPD profiling method in detecting BaP-induced DNA changes was also determined using both replicates R1 and R2 (Fig. 2C and D). This experiment was performed to confirm if extra bands 9-1 and 9-2 appeared in the majority of the organisms exposed to 0.025 (Fig. 2C) and 0.05 (Fig. 2D) mg/L BaP. Results suggested that both bands were very reproducible between individuals and mixtures of *Daphnia*. The frequency of bands 9-1 and 9-2 was calculated to be at least of 95% at 0.025 and 0.05 mg/L BaP. When the same samples were subjected to analysis with primer OPB7, the bands 7-1, 7-2, and 7-3 appeared all together or not at all (data not shown). The frequency of appearing bands at 0.025 and 0.05 mg/L BaP was calculated to be 42 and 87%, respectively. In addition to that, further experiments confirmed that...
that the variation in band intensities was not a consequence of either a variation in the concentration of template DNA within a certain range (data not shown) or a variation in PCR reagent concentration (e.g., Taq DNA polymerase) since a master mix was performed.

An accessory experiment designed to determine the effects of BaP exposure on subsequent generations of *D. magna* was performed by comparing profiles of pooled DNA from the offspring (last generation) of BaP-exposed animals against the banding pattern obtained with maternal DNA (Fig. 2E). Results indicate that extra bands (7-1, 7-2, 7-3) present in maternal DNA profiles were not visible in neonatal profiles at low concentrations of BaP (0.0125 and 0.025 mg/L) but may appear at higher concentrations (0.05 mg/L, replicate 1). Future work will investigate this possibility in greater detail.

**Comparison of fitness parameters and RAPD profiles**

To compare the sensitivity of the parameters presented in Figure 3, changes in each factor were calculated as a percentage of their control value (set to 100%). All the parameters presented in Figure 3 were measured until *D. magna* were unable to swim but were still alive after 14, 7, and 5 d at 0.05, 0.1, and 0.2 mg/L BaP, respectively. For the other groups (i.e., control, control + DMF, 0.0125, and 0.025 mg/L BaP), the measures were performed throughout the experiment. Changes in RAPD profiles were expressed as reductions in genomic template stability (a qualitative measure reflecting the obvious changes to number and intensity of DNA bands in DNA patterns generated by toxicant-exposed daphnids) in relation to profiles obtained from control *Daphnia*. Before transformation (i.e., control value set to 100%), the genomic template stability was 96.5, 91.3, 63.4, 44.9, 28.6, 46.3, and 53.6% in control, control + DMF, 0.0125, 0.025, 0.05, 0.1, and 0.2 mg/L BaP, respectively. Although *m* and *R* are the most sensitive fitness parameters, changes in DNA profiles are more sensitive than any other population parameter. Interestingly, based on observation, fitness parameters were found to be negatively correlated to BaP concentrations, whereas the genomic template stability followed a reversed Gauss curve (Fig. 3).

**DISCUSSION**

In ecotoxicology, the specific evaluation and environmental monitoring of potentially genotoxic agents would be improved with the development of sensitive and selective methods to detect toxicant-induced alterations in the genomes of a wide
range of biota. Nevertheless, the value of such procedures would be further enhanced by linking molecular/cellular effects to higher order changes such as reductions in Darwinian fitness and declines in species diversity [1–3]. This study evaluates the suitability of a DNA profiling/fingerprinting assay combined with the measurement of parameters at the population level with the objective of better understanding the impact of BaP on D. magna. The RAPD profiles detect alterations to genomic DNA through the use of randomly primed PCR reactions. These effects include changes in oligonucleotide priming sites and variations in the activity of the Taq DNA polymerase. Such effects lead to visible changes in the electrophoretic profiles of RAPD reaction products. Changes include the appearance of extra amplified bands, the apparent disappearance of amplified bands, and the changes in amplified band fluorescence. However, although obvious changes occurred in RAPD profiles obtained from the exposed population, there were no obvious trends among patterns obtained at the different BaP concentrations. New PCR amplification products may reveal a change in the DNA sequence due to mutations (resulting in [a] new annealing event[s]) and/or large deletions (bringing two preexisting annealing sites closer) and/or homologous recombination (juxtaposing two sequences that match the sequence of the primer). Indeed, this method (in fact, the AP-PCR technique) has already been successfully used for the detection of mutations [12,13]. Following exposure to mutagens, DNA replication [27] and error-prone DNA repair [28] are generally implicated in generating mutations (e.g., B-

Fig. 3. Comparison between key fitness parameters and genomic template stability in populations of Daphnia magna exposed to BaP. $I_1$ = age-specific survival, $m_i$ = age-specific fecundity, $r_p$ = net reproductive rate, and $r_e$ = intrinsic rate of natural increase (see materials and methods and results). $\square$, 0 (control); $\square$, 0 (solvent control); $\square$, 0.0125; $\square$, 0.025; $\square$, 0.05; $\square$, 0.1; and $\square$, 0.2 mg/L BaP. * and ** indicate a significant difference from control ($p < 0.05$ and $p < 0.01$, respectively). Error bars represent standard deviation.

The genomic template stability is directly related to the extent of DNA damage and also to the efficiency of DNA repair and replication. For example, a high level of DNA damage does not necessarily decrease the genomic template stability (in comparison to a low level of DNA alterations) because DNA repair and replication may be inhibited due to excessive, lethal actions of the BaP-induced adducts. If the survivability of a population is affected, a toxic effect can be completely inhibited a biological response (e.g., fitness parameters); in contrast, the genomic template stability cannot be completely affected because the induction of DNA damage may not increase linearly (plateau effect) (Fig. 3). Furthermore, since genomic template stability may be related to different kinds of DNA damage, such as DNA adducts, mutations, rearrangements, etc., it would be difficult to anticipate a dose–response relationship.

It is proposed that alterations to RAPD profiles due to genotoxic exposure can be regarded as alterations in genomic DNA template stability and that this qualitative measure of genotoxic effect can be directly compared with changes in key Darwinian fitness parameters. The results from this experiment clearly suggest that genomic DNA template stability can be more sensitive than growth parameters and of at least equal or even greater sensitivity than other measures of fitness such as age-specific survival, age-specific fecundity, net reproductive rate, and the intrinsic rate of population increase. It is also important to note from this series of experiments that RAPD profiling can detect DNA changes earlier than other changes in conventional toxicity assays measuring fitness parameters. After exposing larval Xenopus laevis to BaP, Sadinski et al. [7] suggested that DNA adducts and micronuclei were sensitive measures of sublethal DNA damage as well as possible short-term indicators of indirect effects on fitness parameters. The present study, using a novel and simple approach, also supports this notion in a clonal freshwater invertebrate species.

The growth experiment suggested that Daphnia exposed to 0.1 and 0.2 mg/L BaP had a size significantly reduced. By attaining reproductive maturity at a smaller body size, the Daphnia are able to buffer the impact of lower body growth rate on the age at the first reproduction [33]. Generally, when food is limited, Daphnia generate fewer but larger neonates compared with organisms fed with adequate amounts of food [34]. Thus, the identical size of the neonates at first reproduction plus the fact that growth rates were a good indicator of the intrinsic rate of population increase ($r_e$) [35] suggest that the decrease in size at maturity was more readily ascribed to a direct toxic effect of BaP. In other words, the effects on growth were not due to a limitation in food levels. The data also indicate that D. magna exposed to gradual increases in...
BoP concentrations lengthened the time between the first and second brood. This response is generally known to lessen the impact of adverse effects on survival and the number of offspring at each breeding [36].

The measure of molecular and population parameters present several advantages. First, in ecotoxicology, it is fundamental to accumulate data at different levels of biological organization in order to fully understand the effect of a toxicant on organisms. Second, the measure of some parameters at the population level facilitates the interpretation of the data at the molecular level. For instance, a significant reduction in growth correlates with a significant inhibition in DNA replication, suggesting that the extent of DNA damage may be important in the majority of the cells.

The random nature of the DNA amplification events that form the basis of the RAPD profiling technique has often attracted criticism. The generation of profiles has regularly been considered to exhibit poor reproducibility [37]. However, after suitable optimization of amplification reaction conditions [18] and the judicious choice of oligonucleotide primers for each species-specific DNA template, the assay performs well, even for nonclonal organisms. For example, we successfully applied the RAPD technique to a wide range of species such as Platynereis dumerilii (worm), Mytilus edulis (mussel), Palmaria palmata (green macroalgae), Enteromorpha intestinalis (green macroalgae), Escherichia coli (bacteria), Renibacterium salmoninarum [38] (bacteria), together with calf thymus and human placental tissue (unpublished manuscript). While the RAPD technique clearly shows promise in the detection of pollutant-induced DNA effects, a great deal of further experimentation and validation is required. Future work will focus on the determination of sensitivity and selectivity of the RAPD technique by comparison with preexisting DNA analysis methodologies. In particular, the performance of RAPD will be compared with 32P postlabelling for the detection of BoP adducts in Daphnia DNA. It is also planned to assess the performance of this assay in the detection of chromosomal aberrations, DNA strand breaks, and DNA sequences containing multiple and defined mutations. The latter will be performed using material obtained from both hypermutating cultured animal cells and from bacterial isolates. Using this approach, it should then be possible to determine the general applicability of this method to the detection of DNA effect in both in vitro and in vivo systems and the specific ability of RAPD to evaluate pollutant-induced genotoxicity in natural populations of invertebrate animals.

In conclusion, the RAPD and AP-PCR techniques show potential as reliable and reproducible assays for genotoxicity. These techniques are therefore being increasingly adopted as sensitive methods for the detection of induced genetic damage at the molecular level in both somatic and germ cells of aquatic organisms (e.g., [16,39]). The present study suggests that, when coupled with the measurement of pollutant-induced effects at higher levels of biological organization, this technique would prove to be a powerful ecotoxicological tool.

Acknowledgement—This work was supported financially by the Marie Curie Grant of the European commission (ERB-4001-GT-97-0136). We wish to thank Brixham Environmental Laboratory, Zeneca, Brixham, United Kingdom, for their help in establishing the D. magna culture.

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