DNA adduct detection: some applications in monitoring exposure to environmental genotoxic chemicals

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Postlabelling Methods for Detection of DNA Adducts
Ed. D.H.Phillips, M. Castegnaro & H. Bartsch
Lyon, International Agency for Research on Cancer

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Summary. In the assessment of genotoxic risk factors in the environment, the measurement of DNA adducts in aquatic organisms and in plants may have considerable implications. Using ^{32}P-postlabelling, we have detected DNA adducts in the liver of carp (*Chondrostoma nasus*) from the River Rhône (France), both downstream and upstream from a polychlorinated biphenyl incineration plant. Some of the DNA adducts were specific to downstream fish, suggesting a differential pattern of exposure. We have also detected DNA damage in needles in a declining spruce forest. We found that, in the declining forest, the amounts of DNA adducts increase in relation to the degree of damage to the needles whereas, in a healthy forest, the levels of DNA adducts were low. We have also found DNA adducts in the leaves of hops grown in fields where heptachlor residues persisted.

Introduction

There is now considerable evidence that, in humans and in animals, many chemicals or their metabolites bind covalently to DNA *in vivo*, forming DNA adducts (Randerath *et al.*, 1985). In mammals, these DNA modifications may lead to the genetic alterations necessary for the initiation of carcinogenesis. Thus, detection of DNA adducts in humans is currently used for monitoring exposure to genotoxic compounds. For this purpose, the sensitive ^{32}P-postlabelling assay (Randerath *et al.*, 1981) is a useful tool, since it detects and quantifies DNA adducts resulting from exposure to unknown chemicals. Moreover, in the field of environmental toxicology, DNA adduct detection in wild animals may provide evidence for contamination of the environment by genotoxic chemicals.

In the studies described below, we have analysed hepatic DNA from fish living in the River Rhône and needle DNA from trees growing in a polluted area of the Vosges mountains in France using the ^{32}P-postlabelling assay. We also investigated DNA adduct occurrence in hops grown on heptachlor-contaminated soil.

Material and methods

Male and female carp (*Chondrostoma nasus*) were caught by netting at two locations on the upper Rhône (between Geneva and Lyon), at Pont de Lucey (the upstream site) and at Miribel (the downstream site). Livers were excised and frozen in liquid nitrogen until further processing. Hepatic DNA was isolated according to Dunn and Stich (1986).

Needle DNA from pine trees (*Picea abies*) and leaf DNA from hops (*Humulus lupulus*) were prepared according to Guillemaut and Marechal-Drouard (1992). For fish DNA and hop-leaf DNA, DNA adducts were detected using the ^{32}P-postlabelling method in its nuclease P1-mediated enrichment version, as described by Reddy and Randerath (1986). For needle DNA, adducts were isolated by extraction with 1-butanol (Gupta, 1985; Gallagher *et al.*, 1989).

Results and discussion

Detection of DNA adducts in fish liver

DNA adduct patterns are presented in Figure 1. Several adducts (Nos. 1–4, 6–9 and 11) were found in the hepatic DNA from male carp from both locations (Figure 1A,B). However, adducts 5, 10 and 12 were detected only in Miribel fish (downstream site). For female fish, only one major adduct (No.2) and two minor adducts (Nos. 3 and 4) were detected in Pont de Lucey fish (Figure 1C). These three adducts were also found in the liver DNA from females caught at the downstream site (Miribel), but seven other DNA adducts were specific for this location (see Figure 1D). Thus, our results suggest that fish from the upper Rhône are exposed to genotoxic chemicals (which bind to DNA directly or after metabolic activation) and indicate that there is contamination of the Miribel site.

The analysis of fish DNA using the ^{32}P-postlabelling method has been carried out in a number of field surveys. For example, DNA adduct levels in livers from English sole were higher when the fish sampled were from a PAH-contaminated area (Varanasi *et al.*, 1989). In the same way, Dunn *et al.* (1987) showed that DNA adduct levels in livers of aquarium-raised bullheads were well below the levels determined in fish from polluted rivers. However, Kurelec *et al.* (1989) did not observe any differences in adduct levels with various fish species from polluted versus non-polluted waters. Although many of the results obtained in such studies are only of a qualitative nature (comparison of adducts patterns), they confirm the usefulness of the method for estimating the degree of exposure of fish to genotoxic compounds.

Formation of abnormal nucleotides in plant DNA exposed to xenobiotics in the field

*Detection of DNA adducts associated with conifer (*Picea abies*) forest decline*

Forest decline is an important problem in North America and Europe, where millions of hectares are already damaged. This study concerns a declining spruce forest located at an altitude of 750 metres above sea level in the Donon region of the Vosges mountains in north-eastern France. The main symptom of decline is needle yellowing which usually occurs in one or two-year-old needles, although the needles of the current year appear undamaged. As a consequence of yellowing, spruce trees may lose all needles older

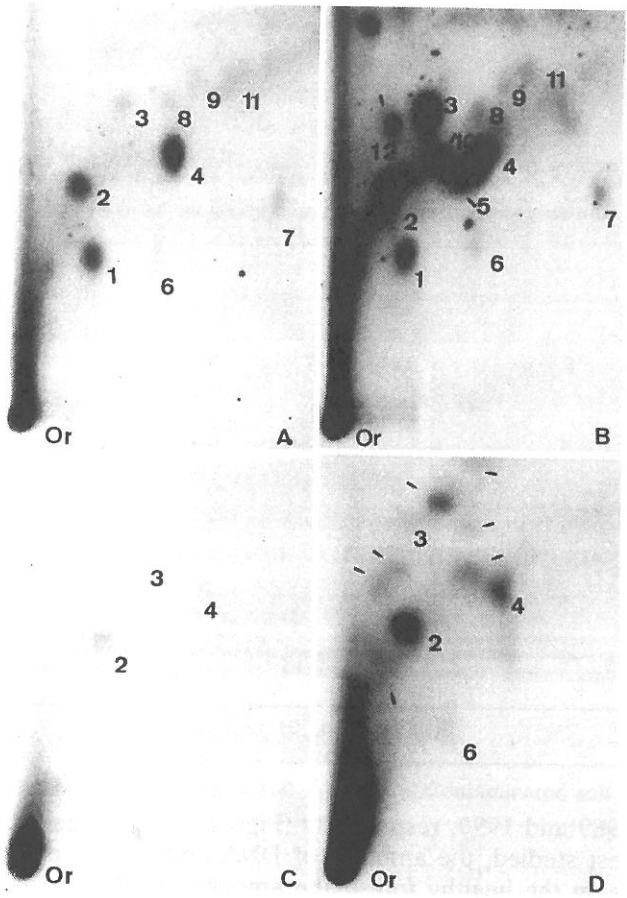

Figure 1. Autoradiograms of PEI-cellulose thin-layer sheets of ^{32}P-postlabelled hepatic DNA digests from carp from Pont de Lucey and Miribel

The arrows point to DNA adducts detected only in Miribel fish. Autoradiograms were exposed for 60 h with one intensifying screen at –80°C. (A) male fish from Pont de Lucey, (B) male fish from Miribel, (C) female fish from Pont de Lucey, (D) female fish from Miribel. Or = origin.

than three or four years, whereas healthy trees retain them for up to seven years. Our results have been obtained with 1989 and 1990 needles collected in July 1991 when they were entirely (1989) or partly yellow (1990) and with three-month old young green needles produced in 1991. A representative analysis of adducts is shown in Figure 2. In the declining forest studied, analysis revealed the presence of four major (Nos. 6–9) and five minor (Nos. 1–5) adducts in the DNA from yellowing needles (Figure 2B,C), whereas only faint adduct spots were detected in the young green needles (Figure 2A). The quantitative analysis revealed that 1989 and 1990 needles contained 21.7 and 10 adducts per 10^9 unmodified nucleotides, respectively, whereas only 3.6 adducts per 10^9 nucleotides were detected in three-month-old young needles. Moreover, when one- or two-year-old green needles from apparently healthy forest were analysed, the level of DNA adducts was very low. The amounts were only 1 and 1.7 adducts per 10^9

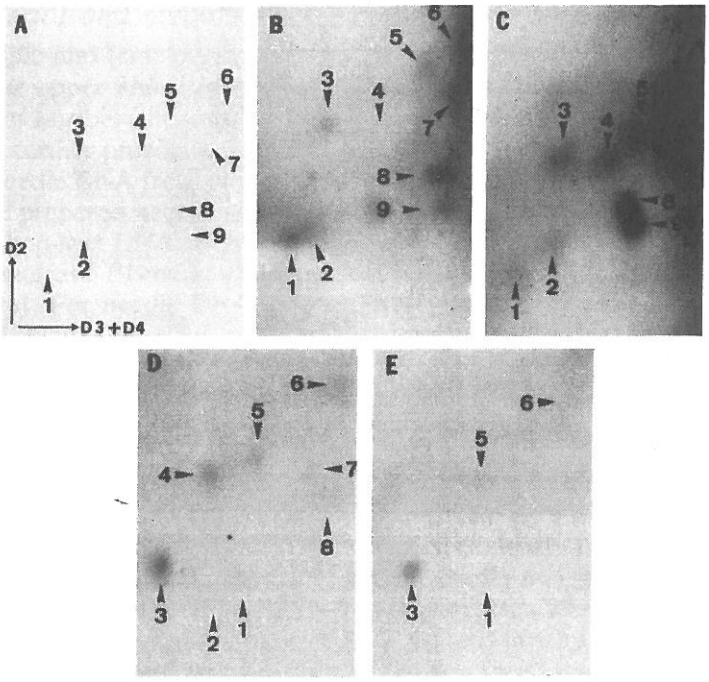

Figure 2. Autoradiograms of TLC sheets of ^{32}P-postlabelled DNA adducts obtained from declining (A, B,C) and healthy (D,E) spruce
(A) Three-month-old green needles of 1991, (B) partially yellow needles of 1990, (C) entirely yellow needles of 1989, (D) green needles of 1990 from healthy trees and (E) green needles of 1989 from healthy trees. Autoradiograms were exposed for 24 h (A,B,C) or 40 h (D,E) with one intensifying screen at −80°C.

nucleotides for 1989 and 1990, respectively (Figure 2D,E). These results show that in the declining forest studied, the amounts of DNA adducts are correlated with needle damage, whereas, in the healthy forest, the amounts of DNA adducts do not change significantly with the age of the needles (Weber-Lotfi *et al.*, 1992).

DNA adduct formation in hop leaves

DNA in leaves from healthy and declining hops was analysed. High levels of heptachlor and heptachlor-epoxide residues have been found in the soil where the hops were declining. This finding prompted us to search for DNA adducts. The DNA adduct patterns obtained are shown in Figure 3. Two major (Nos. 2 and 5) and five faint adducts (Nos. 1, 3, 4, 6 and 7) were observed with healthy hop leaves. In contrast, DNA from declining hop leaves had six major spots (Nos. 1, 2, 4, 5, 9 and 10) and eight faint spots (6, 8 and 11–16). Seven spots were specific for declining hop leaves (9 to 16). Total DNA adduct levels were 3.2 times higher in declining hops (Table 1). A comparison between DNA adducts from several types of plant cells cultured in the presence of heptachlor and from declining hops is in progress in our laboratory.

In conclusion, our data confirm the value of the ^{32}P-postlabelling method for the detection of DNA adducts in fish and demonstrate its use with plants, and thus its applicability to the assessment of contamination of the environment by genotoxic pollutants.

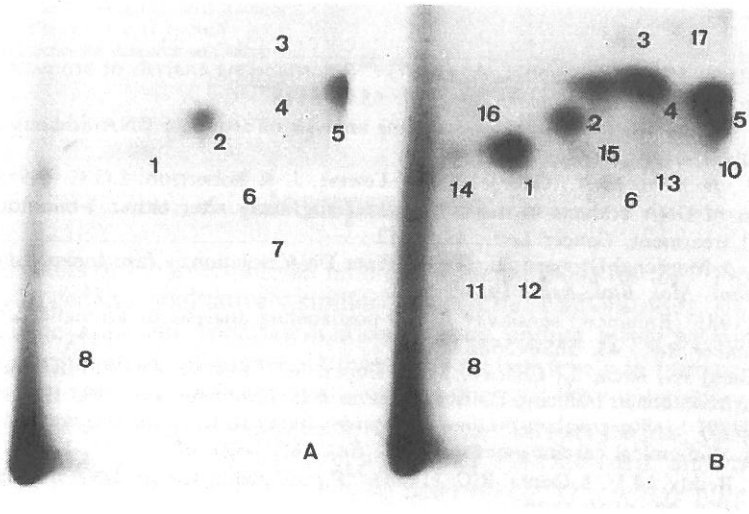

Figure 3. Autoradiograms of TLC sheets from hop leaf DNA

(A) Hops from uncontaminated soil, (B) hops from soil contaminated with heptachlor. Autoradiograms were exposed for 60 h with one intensifying screen at −80°C.

Table 1. Quantification of hop DNA adducts

Adduct no.	Adduct level (per 10^9 nucleotides)	
	Uncontaminated soil	Contaminated soil
1	0.630	3.140
2	1.450	2.500
3	0.300	0.920
4	0.900	2.970
5	2.500	2.970
6	0.400	0.890
7	0.240	nd
8	0.720	1.150
9	0	1.460
10	0	3.260
11	0	0.580
12	0	0.720
13	0	1.270
17	0	1.150
Total adducts	7.1	22.6

Acknowledgements

This work was supported by grants from the Université Louis Pasteur, Strasbourg, the Région Alsace, the Centre National de la Recherche Scientifique, and the Ministère de la Recherche et de la Technologie (Action Toxicologie).

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