Environmental Health Perspectives
Vol 90, pp. 111-116, 1991

Carcinogen Adducts As an Indicator for the Public Health Risks of Consuming Carcinogen-Exposed Fish and Shellfish

by Bruce P. Dunn*

A large variety of environmental carcinogens are metabolically activated to electrophilic metabolites that can bind to nucleic acids and protein, forming covalent adducts. The formation of DNA-carcinogen adducts is thought to be a necessary step in the action of most carcinogens. Recently, a variety of new fluorescence, immunochemical, and radioactive-postlabeling procedures have been developed that allow the sensitive measurement of DNA-carcinogen adducts in organisms exposed to environmental carcinogens. In some cases, similar procedures have been developed for protein-carcinogen adducts.

In an organism with active metabolic systems for a given carcinogen, adducts are generally much longer lived than the carcinogens that formed them. Thus, the detection of DNA- or protein-carcinogen adducts in aquatic foodstuffs can act as an indicator of prior carcinogen exposure. The presence of DNA adducts would, in addition, suggest a mutagenic/carcinogenic risk to the aquatic organism itself. Vertebrate fish are characterized by high levels of carcinogen metabolism, low body burdens of carcinogen, the formation of carcinogen-macromolecule adducts, and the occurrence of pollution-related tumors. Shellfish, on the other hand, have low levels of carcinogen metabolism, high body burdens of carcinogen, and have little or no evidence of carcinogen-macromolecule adducts or tumors. The consumption of carcinogen adducts in aquatic foodstuffs is unlikely to represent a human health hazard. There are no metabolic pathways by which protein-carcinogen or DNA-carcinogen adducts could reform carcinogens. Incorporation via salvage pathways of preformed mucleoside-carcinogen adducts from foodstuffs into newly synthesized human DNA is theoretically possible. However, absolute levels of nucleoside-carcinogen levels in aquatic foodstuffs from polluted areas are very low, and the incorporation of preformed adducts into DNA is strongly discriminated against by the fidelity of DNA synthesis.

Introduction

The exposure of aquatic organisms to carcinogenic chemicals has long been recognized. Considerable attention has been paid to the possibility of cancer in aquatic species as a result of exposure to environmental contaminants (*1*). However, less attention has been paid to the direct public health consequences of eating carcinogen-contaminated aquatic foodstuffs. Recently, the discovery of high tumor frequencies in certain wild fish populations has focused public attention on carcinogen contamination of the aquatic environment and heightened awareness of the potential problems of consuming carcinogen-contaminated fish. The potential magnitude of the problem is shown by recent risk estimates, which have indicated that people eating sports fish from the Great Lakes may be exposed to a carcinogenic risk from pesticides and PCBs that is orders of magnitude higher than their risk from drinking Great Lakes water (*2*).

A variety of chemical carcinogens are actively metabolized by aquatic species. Metabolism of carcinogens typically generates electrophilic metabolites that can bind to nucleic acids and protein, forming covalent adducts. Exposure of an aquatic organism to a metabolizable carcinogen thus has the potential for contaminating an aquatic foodstuff not only with the carcinogen itself, but also with its metabolites and with adducts of the metabolites with cellular macromolecules. These metabolites and adducts must be independently considered in assessing the potential health dangers of eating the organism. On the positive side, adducts and metabolites may be useful in assessing carcinogen exposure of aquatic organisms. This paper explores how adduct measurements can be used in assessing the exposure of fish populations to carcinogens. Attention is focused on DNA adducts of PAHs (polycyclic aromatic hydrocarbons). The general remarks, however, are likely to be applicable to other carcinogens and to carcinogen adducts to proteins.

PAHs in Foodstuffs

The original concern over PAHs in foodstuffs was focused on the contamination of food by smoking or barbecuing (*3*) and whether or not this might be linked to the high rates of stomach cancer in areas such as Iceland where smoked foods compose an important part of the traditional diet (*4*). This concern continues to be expressed; a recent example is the possible relationship between native smoked meat and the high incidence of stomach cancer in Nigeria (*5*). Kramers has recently evaluated the potential human intake and risks of PAHs in the diet and in air pollution (*6*). The estimated PAH intake from the diet exceed PAH in

*British Columbia Cancer Research Center, Vancouver, BC, Canada V5Z 1L3.

take from air; however, the risk from the diet was estimated to be less than the risk from air (*6*). An analysis of the average Swedish diet has indicated that cereals, vegetables, and oils are the major sources of dietary PAHs (*7*). Such analyses, however, do not take into account food preferences in individuals, which could lead to deviations from the average. While there are no good epidemiological studies linking PAH-contaminated foodstuffs to human cancer, the fact that PAHs are powerful carcinogens in animals has led public health authorities in some countries to enact regulations limiting their concentrations in foodstuffs. Similarly, widely publicized dietary recommendations for the reduction of cancer risk have suggested limiting the consumption of smoked food products (*8*).

Reports of PAH carcinogens in edible aquatic organisms date back at least three decades to the work of Cahnmann and Kuratsune (*9*), who measured a number of carcinogens in the tissue of oysters collected from a polluted environment. A survey of PAH carcinogens in fresh and tinned commercial seafoods has indicated that while PAH levels in vertebrate fish are low or not detectable, appreciable levels of PAHs are found in shellfish and in crustaceans such as crab and lobsters (*10*). In addition to such contamination of widely distributed foodstuffs, people may be exposed to carcinogens in noncommercial fish or shellfish harvested by sports fisherman, often from heavily polluted waters. PAH contamination is not limited to the animal kingdom, as PAHs have also been detected in seaweeds (*11*). PAH contamination of aquatic food resources thus appears to be widespread, and public consumption of PAH-contaminated seafoods appears to represent an appreciable exposure to these carcinogens (*12*).

Effects of PAHs on Aquatic Organisms

In the past, most work on carcinogen levels in aquatic organisms has concentrated on the problems of chronic or catastrophic contamination of the marine environment by petroleum products. The main focus of the work was the acute and chronic toxicity of pollution to aquatic organisms themselves, and little if any attention was paid to the public health aspects of carcinogen-contaminated seafoods. Much of this earlier work on PAHs in the aquatic environment has been reviewed by Neff (*13*) and by Stegeman (*14*).

More recently, studies have begun to examine the possible role of pollution-associated PAH as a causative agent for tumors in wild fish populations (*1*). The best-studied example of carcinogen induction of fish tumors is the case of liver tumors in bottom-dwelling marine flatfish in Washington State. Tumors appear to be related to sediment levels of PAHs (*1,15–17*). In the fresh water environment, brown bullheads from a variety of PAH-contaminated areas such as the Black, Buffalo, and Fox rivers have been reported to have an elevated incidence of liver tumors (*1*). Bile from such bullheads exhibits fluorescent metabolites resulting from the metabolism of PAHs (*18*). Baumann has suggested that PAHs are the most likely carcinogens to be responsible for the high rate of liver tumors in brown bullheads in contaminated environments (*19*).

For invertebrates, a large number of studies have shown a relationship between high levels of PAHs in contaminated environments and the accumulation of substantial body burdens of PAHs. However, there is a general consensus that there is no good evidence for any relationship between environmental PAHs and invertebrate neoplastic diseases (*1*).

Adduct Formation and Methods for Measuring Adducts

If an environmental carcinogen is not metabolized, it tends to accumulate in organisms until a steady-state body burden is reached at the point that excretion of the compound equals the rate of uptake from the environment. If metabolism takes place, its effect is typically to form hydrophilic metabolites that are rapidly excreted and tend not to accumulate in the body. This sharply lowers or even eliminates the steady-state body burden of the parent compound. Thus, from the point of view of carcinogen contamination of edible organisms, metabolism is good. From the point of view of an organism encountering environmental carcinogens, carcinogen metabolism is usually bad. Reactive electrophilic intermediates formed during the metabolism of carcinogens can react with cellular macromolecules, forming covalent adducts. The formation of DNA-carcinogen adducts is thought to be a necessary step in the action of most carcinogens (*20*).

There has been extensive research directed toward measuring DNA-carcinogen adducts. Levels of adducts in tissues of carcinogen-exposed animals are low, with typical DNA modification levels being less than one adducted nucleotide per million normal nucleotides. Until recently, adducts could only be measured in a laboratory situation, using radioactively labeled carcinogens. A variety of new fluorescence, immunochemical, and radioactive-postlabeling procedures have, however, now been developed that allow the sensitive measurement of DNA-carcinogen adducts in organisms exposed to environmental carcinogens (*21*). In some cases, similar procedures have been developed for protein-carcinogen adducts. These new techniques are beginning to be used with human subjects in investigations commonly termed "biochemical epidemiology" or "molecular epidemiology" (*21–25*). Similar approaches can be applied to wild animal populations exposed to environmental carcinogens.

Table 1 lists some of the characteristics of three different classes of procedures for measuring DNA-carcinogen adducts in isolated DNA. If a carcinogen forms a fluorescent adduct, then sensitive procedures for measuring the adduct by fluorescence of native or degraded DNA may be developed. This technique has been applied to PAH adducts, but it is not widely applicable to adducts of other carcinogens. Immunoassay procedures have a similar sensitivity, but are more generally applicable. However, such assays depend on the availability of well-characterized monoclonal or polyclonal antibodies, which can be difficult and time-consuming to develop. Furthermore, immunoassays require chemical synthesis of the adduct to act as an antigen in the

Table 1. Characteristics of methods for detecting DNA-carcinogen adducts.

Procedure	Typical amount DNA needed, μg	Typical sensitivity, nmole adduct/mole normal nucleotides	Applicability to different carcinogens
Fluorescence	1000	10–100	Narrow
Immunoassay	1000	100	Broad
^{32}P-postlabeling	10	1	Broad

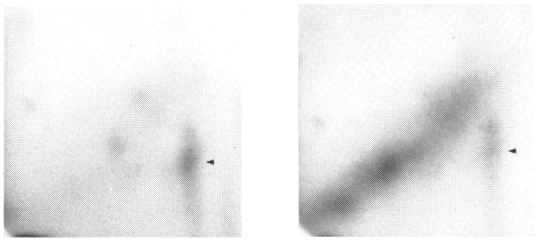

FIGURE 1. ^{32}P-postlabeling detection of DNA adducts in brown bullheads. DNA, 2.5 μg, from the livers of brown bullheads either raised in aquariums or caught in the Buffalo River were analyzed by the nuclease P1 version of the ^{32}P-postlabeling assay, as described elsewhere (*35, 36*). Adducts separated on thin layer chromatograms were detected by autoradiography for 24 hr at room temperature. Arrows indicate background spots not related to environmental contamination.

raising of the antibody, making it impossible to look for adducts of an unknown structure. For maximum sensitivity both immunoassays and fluorescence assays require milligram or greater quantities of DNA, requiring the availability of gram amounts of tissue. Postlabeling assays (*21,26*) depend on enzymatic hydrolysis of DNA to nucleotides, labeling of nucleotides with ^{32}P, and chromatography for the separation and measurement of adducts. Postlabeling procedures typicallly require 10 μg or less of DNA, allowing their use with milligram amounts of tissues.

PAH Metabolism, Adduct Formation, and Body Burden in Vertebrates

Early work on PAH metabolism in marine organisms has been reviewed by Stegeman (*14*). Recent research has begun to delineate subtle details of carcinogen metabolism in fish cells in culture (*27*) and to investigate the metabolism of carcinogens and other foreign compounds in wild fish (*28*). In vertebrate fish, PAH metabolism is generally very rapid. In at least some species of fish, ingested PAHs are extensively metabolized in the intestine, and only a fraction of the applied dose reaches the circulation (*29*). When top minnows were exposed to waterborne radiolabeled benzo(*a*)pyrene, 64 to 70% of the labeled material taken up in a 24-hr exposure was already in the gallbladder or gut, indicating rapid metabolism and excretion of the carcinogen (*30*).

Consistent with the effective metabolism of PAHs by fish, DNA and protein adducts of benzo(*a*)pyrene were readily detected in English sole injected with benzo(*a*)pyrene (*31*). For an equivalent dose of benzo(*a*)pyrene, levels of DNA adducts in English sole and starry flounder were higher than in rats (*32*). When bluegill sunfish were exposed to benzo(*a*)pyrene, fluorescent techniques indicated the presence of both DNA and protein adducts (*33*). Similarly, in laboratory experiments using radioactive carcinogens, English sole exposed to sediments contaminated with aromatic hydrocarbons and radiolabeled benzo(*a*)pyrene readily formed carcinogen-macromolecule adducts (*34*). In this study, steady-state levels of benzo(*a*)pyrene adducts in tissue were reached after approximately 4 days.

Field studies of carcinogen adducts in organisms from chemically contaminated environments are in their infancy. Using ^{32}P-postlabeling, we have recently examined DNA from the livers of brown bullheads from the Buffalo and Detroit rivers, and compared these samples with DNA from bullheads raised in clean aquariums (*35*). Samples were analyzed by both the nuclease P1 and HPLC enrichment versions of the ^{32}P-postlabeling assay (*36*), with selective chromatography to isolate aromatic DNA adducts. Adducts show up as black spots or zones on autoradiograms of thin-layer chromatograms (Fig. 1). Aquarium-raised bullheads exhibited background spots, which may represent either endogenous adducts or adduction from common environmental contaminants. Fish from polluted areas showed, in addition, a strong diagonal radioactive zone, which we interpret as resulting from multiple overlapping adduct spots. The chromatographic and enzymatic behavior of the adducts is consistent with their identification as multiple DNA adduct species arising from mixtures of PAHs, although this identification has as yet not received any direct confirmation (*35*). Also using ^{32}P-postlabeling, Reichert has reported the presence of a similar zone of putative aromatic DNA adducts in DNA from the livers of marine flatfish from polluted areas (*37*).

Consistent with the high metabolic capability of vertebrate fish for PAHs, body levels of PAHs in fish appear to be either very low or not detectable (*1*). Even in fish from the heavily polluted Black River, levels of lower molecular weight aromatic hydrocarbons were orders of magnitude below the levels in sediments, and higher molecular weight, carcinogenic PAHs were undetectable (*38*).

PAH Metabolism, Adduct Formation, and Body Burden in Shellfish

The consensus from a number of investigations is that invertebrates have only a marginal capability for metabolizing and eliminating PAHs (*1,14*). For example, the half-life of benzo(*a*)pyrene in environmentally contaminated mussels has been reported to be greater than 2 weeks (*39*). A large proportion of the metabolism that does takes place in shellfish does not appear to proceed via the action of cytochrome P-450 and produces quinones rather than diolepoxides as a major product (*40*). Such metabolites do not have the biochemical characteristics necessary for DNA adduct formation. Consistent with this, it has been reported that mussels cannot activate benzo(*a*)pyrene to mutagenic metabolites (*41*). A similar situation may hold true for aromatic amine carcinogens. It has been shown that carcinogen metabolism in carp liver microsomes is similar to that of mammalian species, but metabolism in mussels is different and does not involve cytochrome P-450 monooxygenases (*42*). Consistent with the low rate of PAH metabolism in shellfish, a variety of bivalve species have been repeatedly found to contain high levels of pollution-associated PAHs (*1*).

To examine the possibility of adduct formation in wild shellfish from PAH-polluted areas, we have performed ^{32}P-postlabeling analysis on DNA from mussels (*Mytilus edulis*) from the outer harbor of Vancouver, Canada. Clean mussels were from foreshore rocks at a swimming beach, while polluted mussels were growing on creosoted pilings approximately 500 m from the clean samples. PAH levels in mussels were not analyzed, but we

have previously shown consistently elevated levels of PAH carcinogens in mussels growing in creosote-contaminated areas (*11,43,44*). Figure 2 shows autoradiograms of chromatograms derived from DNA of the foot, mantle, and gill of mussels from both areas. Chromatograms exhibited a complex pattern of faint spots that appeared to be tissue specific. However, there were no major differences in the pattern or intensity of the spots between mussels from the clean and from the PAH-contaminated area. This indicates that, at least in this preliminary examination, adduct formation in mussels from environmental PAH seems unlikely. Further investigations in which other tissues such as the metabolically active digestive gland (*14,40*) are examined are clearly warranted before a firm conclusion can be reached about the possibility of PAH adducts in shellfish.

Adducts As Indicators of Environmental Contamination

The presence of DNA adducts of a given carcinogen in the tissue of an aquatic organism provides an unequivocal indication of prior carcinogen exposure of the organism. The exact level of adducts in an organism in a given contaminated environment, however, is likely to be a complex function of such factors as the organisms' age and sex, the time of year, and environmental factors such as food availability and water temperature, which can modify carcinogen metabolism (*45*). It is therefore likely to prove difficult to demonstrate a consistent quantitative relationship between adduct levels in organisms and environmental contamination.

The interpretation of adduct levels in aquatic organisms is further complicated by the scarcity of knowledge about the lifetime of adducts in marine species. In mammals, PAH adduct repair and removal typically takes place with a half-life of days (*46*); however, adducts of some carcinogens have been detected months after exposure (*47*). In fish, there are indications that the DNA repair processes that remove adducts are less active than in mammalian species, which would lead to the prediction of long life times for environmentally caused adducts (*48*). Consistent with this, Varanasi has found that DNA adducts of benzo(*a*)pyrene were essentially unrepaired in English sole over a period of 2 weeks (*31*). Recent research has suggested that not all adducts in mammalian DNA are repaired at the same rate (*47*). If this holds true for fish, an aquatic organism chronically exposed to low levels of a carcinogen might be expected to remove repairable adducts as quickly as they form, but to gradually accumulate unrepairable or slowly repairable adducts. It is these accumulated adducts that are likely to be detected in monitoring programs examining wild fish. In view of this, it seems likely that adducts will have a long half-life in fish and that pollution-induced adduct levels will represent not so much acute exposure to carcinogens, but the typical contamination levels in the aquatic environment over a period of weeks or months.

Adducts As Indicators of Public Health Dangers

Adduct analysis is generally more costly and difficult than direct analysis for carcinogens, and for this reason if no other it seems unsuited as a testing method to determine if an edible aquatic organism is at risk for carcinogen contamination. However, the presence in an organism of adducts of a given carcinogen clearly indicates past exposure to the carcinogen and raises the possibility of ongoing contamination with the carcinogen. In addition, the presence of adducts will generally suggest the presence of carcinogen metabolites, for which there are generally no good analytical techniques available and which may pose an appreciable human health hazard. For public health purposes therefore, it seems prudent that where analysis has shown the presence of carcinogen adducts, an aquatic organism should be regarded as being carcinogen contaminated.

Without further information, the absence of adducts cannot indicate the absence of carcinogens. The absence of adducts may simply be due to the failure of the species in question to meta

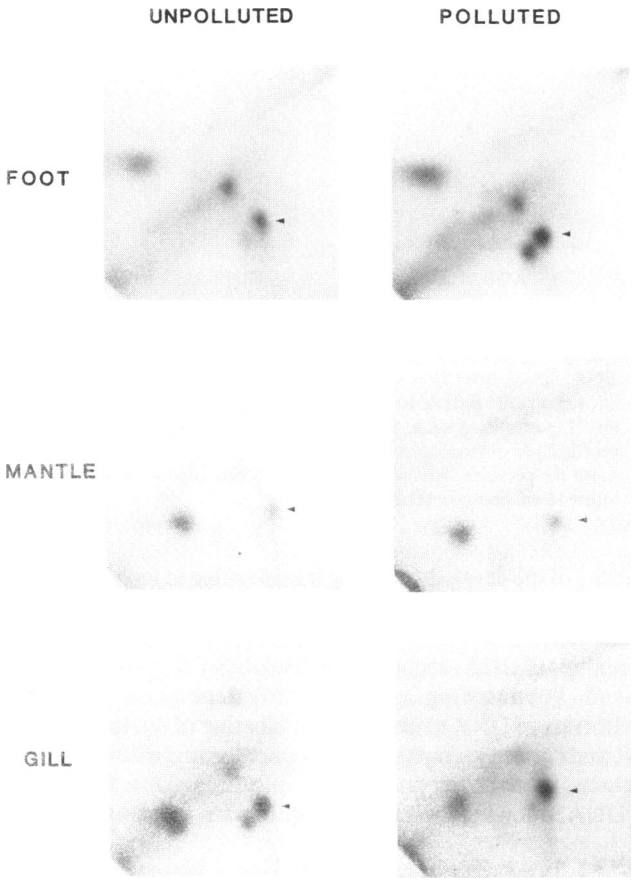

FIGURE 2. ^{32}P-postlabeling detection of DNA adducts in mussels. DNA, 2. 5 μg samples, from the foot, mantle, or gills of mussels from a clean and a creosote-contaminated area in the outer Vancouver harbor were analyzed by the nuclease P1 version of the ^{32}P-postlabeling assay, as described elsewhere (*35, 36*). Adducts separated on thin layer chromatograms were detected by autoradiography for 65 hr at room temperature. Arrows indicated background spots not related to environmental contamination.

bolize a carcinogen to reactive species. Furthermore, even in organisms capable of carcinogen metabolism, metabolism and adduct formation are frequently tissue specific. The failure to detect adducts in a given tissue may simply indicate that the wrong tissue was chosen. Only when adducts of a specific carcinogen are known to occur in a specific tissue of a specific species can their absence be used as evidence for the absence of the carcinogen.

Public Health Dangers of Adducts

Carcinogen adducts are the products of the covalent binding of highly reactive species with cellular macromolecules. General considerations of chemical kinetics suggest that preformed carcinogen-macromolecule adducts in foodstuffs are thermodynamically unlikely to spontaneously regenerate carcinogens or carcinogen metabolites. The question then rises as to whether carcinogen-adducted protein or DNA as such in edible aquatic organisms could be dangerous.

The majority of carcinogen adducts in the edible tissue of an aquatic organism are likely to be protein adducts. When consumed and digested, these are likely to yield adducted amino acids or peptides that will be either eliminated as such or further broken down by catabolic enzymes. Even if adducted amino acids were activated and incorporated into newly synthesized protein, this would not represent a genetic or carcinogenic threat.

The intestinal digestion of adducted nucleic acids consumed in foodstuffs is likely to yield adducted nucleotides, nucleosides, and bases. Recently, Tierney et al. have demonstrated the rapid excretion of a putative benzo(*a*)pyrene-deoxyguanosine adduct in rats fed DNA adducted with benzo(*a*)pyrene (*49*). Metabolic pathways exist by which preformed nucleotides and bases can be salvaged and converted to nucleoside triphosphates, which can in turn be incorporated into newly synthesized nucleic acids. If adducted nucleotides were to be incorporated into DNA by these pathways, the end result could be genetic damage indistinguishable from that produced by the direct attack of the original carcinogen on DNA. There has been considerable debate over whether mutagenesis (and by extension, carcinogenesis) can occur by such mechanisms (*50*). DNA synthetic processes inherently have high fidelity and discriminate strongly against the incorporation of incorrect nucleotides (*51*). Incorporation is most likely therefore, for nucleotides differing only slightly from the parent nucleotide, either where the base modification does not affect base pairing or where alternate base-pairing modes exist. Even for nucleotides adducted with as little as a methyl group, evidence suggests that incorporation rates into newly synthesized DNA are extremely low relative to normal nucleotides (*50*). For larger adducts, the opinion has been expressed that incorporation is extremely unlikely to take place at all (*50*). On an even more basic level, it can be argued that if a carcinogen-adducted or otherwise abnormal nucleotide is so similar to a normal nucleotide that it can be readily incorporated into DNA, it effectively is a normal nucleotide and is unlikely to cause mutations at the next round of DNA synthesis. An example of this phenomenon is given by the thymidine analog bromodeoxyuridine, which can be incorporated at high rates into DNA with little or no effect on cells. Only a very small fraction of a carcinogen entering an organism is converted into DNA adducts, and absolute levels of adducts in tissues of edible organisms are low. Overall, therefore, the presence of adducts in seafood does not seem likely to directly pose a danger to human health.

Table 2. Characteristics of vertebrate fish and shellfish with respect to polycyclic aromatic hydrocarbons.

		Body burden		Carcinogenic risk to	
Species	Metabolism	Parent carcinogen	Adducts, metabolites	Fish	Humans
Vertebrate fish	High	Low	High	High	Low
Shellfish	Low	High	Low	Low	High

Conclusions

Table 2 summarizes the relationship between PAH metabolism, carcinogen body burdens, and genetic risk for vertebrate fish and shellfish. Paradoxically, the same metabolic processes that activate environmental carcinogens to DNA-reactive forms are responsible for the efficient elimination of the parent carcinogen from an organism. An aquatic organism with a high level of carcinogen metabolism may be at risk from the carcinogen but may be safe to eat, as steady-state carcinogen levels in the tissue are low. Conversely, an organism that does not metabolize a carcinogen may not itself be at risk, but may accumulate substantial tissue levels of carcinogens and may be a danger to public health. In aquatic environments contaminated by PAH carcinogens, vertebrate fish have active carcinogen metabolism, high DNA-carcinogen adduct levels, but low tissue levels of the parent carcinogens. Shellfish, on the other hand, are characterized by low carcinogen metabolism, low DNA-carcinogen adduct levels, and high tissue levels of the parent carcinogens. In keeping with this pattern, there is compelling evidence for the carcinogenicity of PAHs to vertebrate fish, but little or no evidence of their carcinogenicity to shellfish.

This work was supported by grant A3403 from the Natural Sciences and Engineering Research Council of Canada. The expert technical assistance of Daniel Twa is gratefully acknowledged.

REFERENCES

1. Mix, M. C. Cancerous diseases in aquatic animals and their association with environmental pollutants: a critical literature review. Mar. Environ. Res. 20: 1–141 (1986).
2. Bro, K. M., Sonzogni, W.C., and Hanson, M. E. Relative cancer risks of chemical contaminants in the Great Lakes. Environ. Manag. 11: 495–505 (1987).
3. Lo, M. T., and Sandi, E. Polycyclic aromatic hydrocarbons (polynuclears) in foods. Residue Rev. 69: 35–86 (1978).
4. Dungal, N. The special problem of stomach cancer in Iceland with particular reference to dietary factors J. Amer. Med. Assn. 178: 789–798 (1961).
5. Alonge, D. O. Carcinogenic polycyclic aromatic hydrocarbons (PAH) determined in Nigerian kundi (smoke-dried meat). J. Sci. Food Agric. 43: 167–72 (1988).
6. Kramers, P. G. N., and Van der Heijden, C. A. Polycyclic aromatic hydrocarbons (PAH): carcinogenicity data and risk extrapolations. Toxicol. Environ. Chem. 16: 314–351 (1988).
7. Friberg, L. The GESAMP evaluation of potentially harmful substances in fish and other seafood with special reference to carcinogenic substances. Aquat. Toxicol. 11: 379–93 (1988).

8. National Research Council Committee on Diet, Nutrition and Cancer. Diet, Nutrition, and Cancer. National Academy Press, Washington DC, 1982.
9. Cahnmann, H. J., and Kuratsusne, M. Determination of polycyclic aromatic hydrocarbons in oysters collected in polluted water. Anal. Chem. 29: 1312–1317 (1957).
10. Dunn, B. P., and Fee, J. Polycyclic aromatic hydrocarbon carcinogens in commercial seafoods. J. Fish Res. Board Can. 36: 1469–1476 (1979).
11. Dunn, B. P. Polycyclic aromatic hydrocarbons in marine sediments, bivalves, and seaweeds: analysis by high-pressure liquid chromatography. In: Polycyclic Aromatic Hydrocarbons (A. Bjorseth and A. J. Dennis, Eds.), Battelle Press, Columbus, OH, 1980, pp. 367–377.
12. Dunn, B. P. Polycyclic Aromatic hydrocarbons (PAH). In: Carcinogens and Mutagens in the Environment, Vol. 1, Food Products (H. F. Stich, Ed.), CRC Press, Boca Raton, FL, 1982, pp. 175–183.
13. Neff, J. M. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment: Sources, Fates, and Biological Effects. Applied Science Publishers, London, 1979.
14. Stegeman, J. J. Polycyclic aromatic hydrocarbons and their metabolism in the marine environment. In: Polycyclic Hydrocarbons and Cancer, Vol. 3 (H. V. Gelboin and P. Ts'O Eds.), Academic Press, New York, 1981, pp. 1–60.
15. Malins, D. C., McCain, B. B., Brown, D. W., Varanasi, U., Krahn, M. M., Myers, M.S., and Chan, S. L. Sediment-associated contaminants and liver diseases in bottom-dwelling fish. Hydrobiologica 149: 67–74 (1987).
16. Malins, D. C., McCain, B. B., Meyers, M. S., Brown, D. W., Krahn, M. M., Roubal, W. T., Schiewe, M. H., Landahl, J. T., and Chan, S. L. Field and laboratory studies of the etiology of liver neoplasms in marine fish from Puget Sound. Environ. Health Perspect. 71: 5–16 (1987).
17. Malins, D. C., McCain, B. B., Landahl, J. T., Myers, M. S., Krahn, M. M., Brown, D. W., Chan, S. L., and Roubal, W. T. Neoplastic and other diseases in fish in relation to toxic chemicals: an overview. Aquat. Toxicol. 11: 43–67 (1988).
18. Maccubbin, A. E., Chidambaram, S., and Black, J. J. Metabolites of aromatic hydrocarbons in the bile of brown bullheads (*Ictalurus nebulosis*). J. Great Lakes Res. 14: 101–108 (1988).
19. Baumann, P.C., Smith, D. W., and Parland, W. K. Tumor frequencies and contaminant concentrations in brown bullheads from an industrialized river and a recreational lake. Trans. Amer. Fish Soc. 116: 79–86 (1987).
20. Lutz, W. In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogensis. Mut. Res. 65: 289–356 (1979).
21. Santella, R. M. Application of new techniques for the determination of carcinogen adducts to human population monitoring. Mutat. Res. 205: 271–282 (1988).
22. Harris, C. C., Weston, A., Willey, J. C., Trivers, G. E., and Mann, D. L. Biochemical and molecular expidemiology of human cancer: indicators of carcinogen exposure, DNA damage, and genetic predisposition. Environ. Health Perspect. 75: 109–119 (1987).
23. Perera, F. P. The potential usefulness of biological markers in risk assessment. Environ. Health Perspect. 67: 141–145 (1987).
24. Perera, F. P. Molecular cancer epidemiology: a new tool in cancer prevention. J. Natl. Cancer Inst. 78: 887–898 (1987).
25. Perera, F. P. The significance of DNA and protein adducts in human biomonitoring studies. Mutat. Res. 205: 255–269 (1988).
26. Watson, W. P. Post-labeling for detecting DNA damage. Mutagenesis 2: 319–331 (1987).
27. Smolarek, T. S., Morgan, S. L., Moynihan, C. G., Lee, H., Harvey, R. G., and Baird, W. M. Metabolism and DNA adduct formation of benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene in fish cell lines in culture. Carcinogenesis 8: 1501–1509 (1987).
28. Varanasi, U., Stein, J. E., Nishimoto, M., Reichert, W. L., and Collier, T. K. Chemical carcinogenesis in feral fish: uptake, activation and detoxification of organic xenobiotics. Environ. Health Perspect. 71: 155–170 (1987).
29. Van Veld, P.A., Patton, J. S., and Lee, R. F. Effect of preexposure to dietary benzo(a)pyrene (BP) on the first-pass metabolism of BP by the intestine of toadfish (*Opsanus tau*): in vivo studies using portal vein-catheterized fish. Toxicol. Appl. Pharmacol. 92: 255–265 (1988).
30. Goddard, K. A., Schultz, R. S., and Stegeman, J. J. Uptake, toxicity, and distribution of benzo(a)pyrene and monooxygenase induction in the topminnows *Poeciliopsis monacha* and *Poeciliopsis lucida*. Drug Metab. Disp. 15: 449–455 (1987).
31. Varanasi, U., Stein, J. E., and Hom, T. Covalent binding of benzo(a)pyrene to DNA in fish liver. Biochem. Biophys. Res. Commun. 103: 780–787 (1981).
32. Varanasi, U., Nishimoto, M., Reichert, W. L., and Eberhart, B. Comparative metabolism of benzo(a)pyrene and covalent biding to hepatic DNA in English sole, Starry founder, and rat. Cancer Research 46: 3817–3824 (1986).
33. Shugart, L., McCarthy, J., Jimenez, B., and Daniels, J. Analysis of adduct formation in the bluegill sunfish (*Lepomis machrochirus*) between benzo(a)pyrene and DNA of the liver and hemoglobin of the erythrocyte. Aquat. Toxicol. 9: 319–325 (1987).
34. Stein, J. E., Hom, T., Casillas, E., Friedman, A., and Varansi, U. Simultaneous exposure of English sole (*Parophrys vetulus*) to sediment-associated xenobiotics: Part 2. Chronic exposure to an urban estuarine sediment with added ^{3}H-benzo(a)pyrene and ^{14}C-polychlorinated biphenyls. Mar. Environ. Res. 22: 123–149 (1987).
35. Dunn, B. P., Black, J. J., and Maccubbin, A. ^{32}P-postlabeling analysis of aromatic DNA adducts in fish from polluted areas. Cancer Res. 47: 6543–6548 (1987).
36. Dunn, B. P., and San, R. H. C. HPLC enrichment of hydrophobic DNA-carcinogen adducts for enhanced sensitivity of ^{32}P-postlabeling analysis. Carcinogenesis 9: 1055–1060 (1988).
37. Reichert, W. L., and Varanasi, U. Detection of damage by ^{32}P-analysis of hepatic DNA in English sole from an urban area and in fish exposed to chemicals extracted from the urban sediment. Proc. Amer. Assoc. Cancer Res. 28: 95 (1987).
38. West, W. R., Smith, P. A., Stoker, P. W., Booth, G. M., Smith-Oliver, T., Butterworth, B. E., and Lee, M. L. Analysis and genotoxicity of a PAC (polycyclic aromatic compounds)-polluted river sediment. In: Polynuclear Aromatic Hydrocarbons (M. Cooke and A. J. Dennis, Eds.), Battelle Press, Columbus, OH, 1985, pp. 1395–411.
39. Dunn, B. P. Release of the carcinogen benzo(a)pyrene from environmentally contaminated mussels. Bull. Environ. Contam. Toxicol. 15: 398–401 (1976).
40. Stegeman, J. J. Benzo(a)pyrene oxidation and microsomal enzyme activity in the mussel (*Mytilus edulis*) and other bivalve mollusc species from the western North Atlantic. Mar. Biol. 89: 21–30 (1985).
41. Britvic, S., and Kurelec, B. Selective activation of carcinogenic aromatic amines to bacterial mutagens in the marine mussel *Mytilus galloprovincialia* Comp. Biochem. Physiol. C 85C: 111–114 (1986).
42. Kurelec, B., and Krca, S. Metabolic activation of 2-aminofluorene, 2-acetylaminofluorene and N-hydroxyacetylaminofluorene to bacterial mutagens by mussel (*Mytilus galloprovencialis*) and carp (*Cyprinus carpio*) subcellular preparations. Comp. Biochem. Physiol. C 88C: 171–177 (1987).
43. Dunn, B. P., Stich, H. F. The use of mussels in estimating benzo(a)pyrene contamination of the marine environment. Proc. Soc. Expt. Biol. Med. 150: 49–51 (1975).
44. Dunn, B. P., and Stich, H. F. Monitoring procedures for chemical carcinogens in coastal waters. J. Fish. Res. Board Can. 33: 2040–2046 (1976).
45. Jimenez, B. D., Cirmo, C. P., and McCarthy, J. F. Effects of feeding and temperature on uptake, elimination and metabolism of benzo(a)pyrene in the bluegill sunfish (*Lepomis macrochirus*). Aquat. Toxicol. 10: 47–51 (1987).
46. DiGiovanni, J., Fisher, E. P., and Sawyer, T. W. Kinetics of formation and disappearance of 7,12-dimethylbenz(a)anthracene:DNA adducts in mouse epidermis. Cancer Res. 46: 4400–4405 (1986).
47. Randerath, E., Agraval, H. P., Weaver, J. A., Bordelon, C. B., and Randerath, K. ^{32}P-postlabeling analysis of DNA adducts persisting for up to 42 weeks in the skin, epidermis and dermis of mice treated topically with 7,12-dimethylbenz(a)anthracene. Carcinogenesis 6: 1117–1126 (1985).
48. Walton, D. G., Acton, A. B., and Stich, H. F. DNA repair synthesis following exposure to chemical mutagens in primary liver, stomach, and intestinal cells isolated from rainbow trout. Cancer Res. 44: 1120–11221 (1984).
49. Tierney, B., Martin, C. N., and Garner, R. C. Topical treatment of mice with benzo(a)pyrene or parenteral administration of benzo(a)pyrene diolepoxide (BPDE)-DNA to rats results in faecal excretion of a putative BPDE-deoxyguanosine adduct. Carcinogenesis 8: 1182–1192 (1987).
50. Snow, E. T., and Mitra, S. Do carcinogen modified deoxynucleotide precursors contribute to cellular mutagenesis? Cancer Invest. 5: 119–125 (1987).
51. Radman, M., and Wagner, R. The high fidelity of DNA duplication. Sci. Am. 259(2): 40–46 (1988).