Complex Mixtures and Cancer Risk
Ed. H. Vainio, M. Sorsa & A.J. McMichael
Lyon, International Agency for Research on Cancer

Macromolecular adducts and related biomarkers in biomonitoring and epidemiology of complex exposures

F. Perera,[a] A. Jeffrey,[a] R. M. Santella,[a] D. Brenner,[a] J. Mayer,[a] L. Latriano,[a] S. Smith,[a] T. L. Young,[a] W. Y. Tsai,[a] K. Hemminki[b] and P. Brandt-Rauf[a]

[a] *Columbia University School of Public Health, 60 Haven Avenue, B-109, New York, NY 10032, USA*
[b] *Institute of Occupational Health, 00250 Helsinki, Finland*

Summary. In order to evaluate the potential of biological markers in epidemiology and risk assessment of complex exposures, we review recent studies of macromolecular adducts and oncogene activation in human populations. Results are discussed in terms of the strengths and weaknesses of various study designs in order to identify the most promising approaches for future research.

Introduction

Environmental carcinogenesis (broadly defined to include both lifestyle and non-lifestyle factors) represents an important area for cancer prevention. In particular, mixtures and multiple exposures pose a particular challenge to researchers since humans are rarely exposed to single agents.

Biological markers have a strong potential to contribute to cancer prevention in a number of ways: by permitting early identification of potential risks (hazards) to humans and by providing comparative molecular dosimetry and response data in both humans and experimental animals, facilitating quantitative risk extrapolation between species. Biological markers can also be incorporated into analytic epidemiological studies in such a way as to increase their ability to identify a causal relationship between exposure to specific agents and increased risk of cancer. Very importantly, biomarkers should significantly expand our knowledge of the mechanisms by which individual carcinogens or mixtures exert their effects. Such information would permit more effective intervention and prevention strategies.

Despite this broad potential, the field is at an early stage of development in which many of the newer laboratory methods are still being validated in the laboratory and in small-scale pilot studies. Although the slow pace may cause epidemiologists and risk-assessors to become impatient, this groundwork is essential to the future use of biological markers and molecular epidemiology. At the very least, recent research has demonstrated that a number of methods are adequately sensitive and reproducible for human studies of environmental carcinogenesis and that the

Table 1. Studies of macromolecular adducts in humans

Compound analysed	Exposure source	Biological sample	Population	Reference
Chemical-specific adducts				
N-3-(2-Hydroxyethyl)-histidine; *N*-(2-hydroxyethyl)valine	Ethylene oxide	RBC	Workers, smokers, unexposed	Calleman *et al.*, 1978; Van Sittert *et al.*, 1985; Farmer *et al.*, 1986; Törnqvist *et al.*, 1986; Latriano *et al.*, unpub.
Alkylated Hb	Propylene oxide	RBC	Workers	Osterman-Golkar *et al.*, 1984
4-Aminobiphenyl–Hb	Cigarette smoke	RBC	Smokers, non-smokers	Bryant *et al.*, 1987
Styrene–Hb	Styrene	RBC	Workers	Brenner *et al.*, unpub.
AFB_1–guanine	Diet	Urine	Chinese and Kenyans residing in a high exposure and high and low risk area respectively	Groopman *et al.*, 1985; Autrup *et al.*, 1983
3-Methyladenine	Methylating agents	Urine	Unexposed	Shuker & Farmer, 1988
PAH–DNA	PAH in cigarette smoke, in workplace	WBC, lung tissue, placenta	Lung cancer patients, smokers, workers	Perera *et al.*, 1989b; Shamsuddin *et al.*, 1985; Vähäkangas *et al.*, 1985; Harris *et al.*, 1985; Haugen *et al.*, 1986; Everson *et al.*, 1986, 1988; Perera *et al.*, 1987, 1988a; Garner *et al.*, 1988; Weston *et al.*, 1988; Brenner *et al.*, unpub.
Antibodies to PAH–DNA	PAH in workplace	Serum	Workers	Haugen *et al.*, 1986
O^6-Methyldeoxy-guanosine	Nitrosamines in diet	Oesophageal and stomach mucosa	Chinese and European cancer patients	Umbenhauer *et al.*, 1985; Wild *et al.*, 1986
Cisplatinum–DNA, Cisplatinum–protein	Cisplatin	WBC, Hb, plasma proteins	Chemotherapy patients	Reed *et al.*, 1986; Fichtinger-Schepman *et al.*, 1987; Mustonen *et al.*, 1988; Den Engelse *et al.*, 1988; Perera *et al.*, 1989a
8-Methoxypsoralen–DNA	8-Methoxy-psoralen	Skin	Psoriasis patients	Santella *et al.*, 1988
Styrene oxide–DNA	Styrene	WBC	Workers	Liu *et al.*, 1988
Non-chemical-specific adducts				
Multiple carcinogens by ^{32}P-postlabelling	Betel and tobacco chewing, smoking, industrial exposures, wood smoke	Placenta, lung tissue, oral mucosa, WBC, bone marrow, colonic mucosa	Smokers, workers, volunteers	Everson *et al.*, 1986; Dunn & Stich, 1986; Chacko & Gupta, 1987; Phillips *et al.*, 1986, 1988a, b; Reddy & Randerath, 1987

Source: Perera (1989)
Abbreviations: PAH, polycyclic aromatic hydrocarbon; RBC, red blood cells; WBC, white blood cells; Hb, haemoglobin, AFB, aflatoxin B.

Table 2. Biological markers in smokers and non-smokers

Marker	Smokers	Non-smokers
4-Aminobiphenyl–Hb (pg/g)	154.5 [11.3*] $n = 19$	32.2 [2.9] $n = 18$
SCE (average per metaphase)	10.8 [0.603†] $n = 11$	8.1 [0.47] $n = 10$
Cotinine (ng/ml)	419.2 [47.4*] $n = 10$	0.3 [0.032] $n = 10$

Values are means [S.E.]
*$p = 0.0001$
†$p = 0.002$
Source: Perera *et al.* (1987)

overall approach is feasible. The experience gained to date also allows some recommendations as to the optimal design of future molecular epidemiological studies.

This paper reviews recent developments in the use of macromolecular adducts and complementary markers in biomonitoring and molecular epidemiology, highlighting those which are most pertinent to the evaluation of carcinogenic risks of complex mixtures. Three types of exposures will be evaluated: lifestyle-related (cigarette smoking); occupational; and clinical. The common denominator for the first two is exposure to multiple polycyclic aromatic hydrocarbons (PAHs) and aromatic amines. Because study design dictates the interpretation of results, examples of cross-sectional, longitudinal serial sampling, retrospective and nested case–control studies will be described, with a brief discussion of the advantages and disadvantages of each approach.

As shown in Table 1, considerable research has been directed towards validating macromolecular adducts as markers of the biologically effective dose of carcinogens. Carcinogen–DNA adducts resulting from covalent interaction of electrophilic carcinogens with DNA are considered to be a necessary although not sufficient event in the initiation of chemical carcinogenesis; in certain instances carcinogen–protein

Table 3. Correlation between 4-aminobiphenyl–Hb and ethylene oxide –Hb adducts in smokers and non-smokers

	Smokers (13)	Non-smokers (7)
Ethylene oxide–Hb (nmol/mol)	238 [29.36] (75–393)	18 [3.04] (10–40)
4-Aminobiphenyl–Hb (pmol/mol)	54 [3.88] (30–68)	11 [1.39] (4–7)

Values are means [S.E.], with range in parentheses
$r = 0.83$ smokers and non-smokers, $p < 0.005$
$r = 0.50$ smokers only, $p < 0.005$
Source: Latriano *et al.* (unpublished)

adducts can satisfactorily serve as a surrogate for DNA binding (Miller & Miller, 1981; Weinstein *et al.*, 1984; Wogan & Gorelick, 1985; Harris, 1985; Farmer *et al.*, 1986; Yuspa & Poirier, 1989).

Cross-sectional studies

Most of the research on macromolecular adducts has been cross-sectional in nature, aimed at elucidating the relationship between carcinogen–DNA or carcinogen–protein adducts and estimated exposure to carcinogens. In most cases, multiple exposures or complex mixtures (for example, cigarette smoke, diet, industrial pollution and combination chemotherapy) have been involved.

Cigarette smoke is a classical complex mixture which has been the subject of much molecular research. For example, in a study of 22 smoking and 24 non-smoking volunteers, a battery of markers was evaluated in repeat peripheral blood samples drawn several days apart (Perera *et al.*, 1987) (see Table 2). Environmental histories were obtained by questionnaire. 4-Aminobiphenyl (4-ABP)-haemoglobin (Hb) adducts were found to be more specific to cigarette smoke than DNA adducts of polycyclic aromatic hydrocarbons (PAHs). Unlike PAH–DNA adducts, for which there was a high and variable background, 4-ABP–Hb levels were significantly different ($p = 0.0001$) between smokers and non-smokers. A repeat blood sample (two days later) gave comparable results for 4-ABP–Hb: 139.0 (S.E. 7.9) pg/g for the smokers versus 36.2 (S.E. 5.02) for the non-smokers. Sister chromatid exchanges were also significantly elevated in the smokers and were positively correlated with 4-ABP–Hb. This finding suggests that aromatic amines contribute significantly to the integrated genotoxic effect of cigarette smoke.

Recently samples from a subset of the original group were analysed for ethylene oxide–haemoglobin (EtO–Hb) adducts by the procedure of Törnqvist *et al.* (1986). The levels of these (hydroxyethylvaline) adducts were significantly higher in smokers ($n = 13$) than in non-smokers ($n = 7$) (Latriano, *et al.* unpublished). Ethylene oxide and 4-aminobiphenyl adduct levels were highly correlated ($r = 0.83$) in the smokers and non-smokers combined and in the smokers as a group ($r = 0.50$, $p < 0.005$) (see Table 3). The measured levels of ethylene oxide–haemoglobin adducts were approximately 10-fold higher than those seen by Törnqvist *et al.* (1986) in smokers and non-smokers, possibly as a result of the different standards used (here hydroxyethylvaline instead of ethylene oxide-modified haemoglobin).

This study design has the advantage of simplicity. However, had the differences between the two groups (smokers and non-smokers) been smaller, they might have been missed because of swamping by interindividual variability. For example, a 30% coefficient of variation was seen in levels of 4-ABP–Hb among the smokers, all of whom smoked between one and two packs per day (mean, 1.4 ± 0.4 packs per day).

In contrast to the smoker/non-smoker study, a cross-sectional study of PAH–DNA adducts in long-term employees in an iron foundry in Finland showed a clear dose–response relationship between estimated source-specific exposure to PAHs and levels of adducts ($p = 0.0001$) after adjusting for cigarette smoking and time since vacation (Perera *et al.*, 1988a). Workers were classified as having high exposure

Table 4. PAH–DNA adducts and serum oncogene protein expression in foundry workers and unexposed controls

Ambient exposure (μg BP/m³)	Subject	Daily cigarette consumption	PAH–DNA adduct levels[a] (fmol/μg)			Serum oncogene proteins[a]					
						fes			*ras*		
			Post-vacation	Work 1	Work 2	Post-vacation	Work 1	Work 2	Post-vacation	Work 1	Work 2
	1	0	0.11	0.8	NA	−	−		−	−	
>0.2	2	20	0.13	2.0	NA	+	+		−	−	
(high exposure)	3	0	NA	2.8	NA	−	−		−	−	
0.05–0.2	4	15	0.13	0.36	NA	−	−		−	−	
(medium exposure)	5	20–25	NA	0.32	0.42	−	−	−	−	+	−
	6	15	NA	0.8	0.5	−	−	−	−	−	−
	7	0	0.48	1.28	NA	+	+		−	−	
	8	20	ND	0.4	NA	−	−		−	−	
0.05	9	20	ND	0.38							
(low exposure)	10		ND	1.52							
	11		ND	0.32							
	12		0.08	1.20							
0	9	15		0.08			−			−	
(unexposed controls)	10	10		ND			−			−	
	11	0		0.14			−			−	
	12	0		ND			−			−	
	13	0		ND			−			−	
	14	0		0.3			−			−	
	15	0		ND			−			−	
	16	10		ND			−			−	
	17	15		0.1			−			−	
	18	0		0.1			−			−	

NA, not available; ND, non-detectable

[a] Post-vacation samples were drawn immediately after a one-month vacation; work 1 samples were drawn six weeks after return to work; work 2 samples were drawn more than two months after return to work.

Source: Brandt-Rauf *et al.* (1989a)

to benzo[*a*]pyrene (BP) as a representative PAH (8 h time-weighted average greater than 0.2 μg/m³), medium exposure (0.05–0.2 μg/m³) or low exposure (less than 0.05 μg/m³). However, here also significant interindividual variation was seen in adduct levels in the exposed workers. For example, in 13 foundry workers with comparable medium exposure (0.05–0.2 μg/m³ BP) there was a 20-fold range of PAH–DNA adducts (0.10–2 fmol/μg). Among 18 workers with low exposure (< 0.05 μg/m³ BP) there was a 29-fold range (0.030–0.86 fmol/μg).

Recently, we have investigated whether levels of PAH–DNA adducts (as a marker of biologically effective dose) correlated with activation of oncogenes (as a marker of biological effect), since various PAHs have been shown experimentally to activate the *ras* oncogene (Balmain & Pragnell, 1983; Marshall *et al.*, 1984). For this purpose, we assayed sera from foundry workers and controls, using a modified western blotting technique, for oncogene protein products (*ras*, *fes*, *myc*, *myb*, *sis*, *B-EGF*, *int-1*, *myb*, *src* and *mos*) as previously described (Brandt-Rauf *et al.*, 1989b). The method was able to reveal whether the oncogene was overexpressed, but not whether it had undergone a point mutation. A five-fold increase in protein expression was considered a positive result. As shown in Table 4, one individual in the medium exposure group showed elevated levels of the *ras* oncogene product in one of three serum samples tested. Repeat serum samples from two other individuals in the medium- and high-exposure groups had significantly elevated levels of the *fes* oncogene protein product. Samples from the 10 unexposed controls were uniformly negative. Both *fes* and *ras* serum proteins have previously been detected in lung cancer patients and cigarette smokers (see Brandt-Rauf *et al.*, 1989a, for review). Since foundry workers are also at elevated risk of lung cancer (IARC, 1984), these results suggest that oncogene activation may prove to be a useful early marker for respiratory cancer related to PAH exposure. Obviously, this clue must be followed up by further studies. Two of the three individuals with elevated serum levels of oncogene protein products were smokers of approximately one pack of cigarettes per day. However, it is likely that the major contribution of PAHs to their body burden was from workplace exposure, since daily intake of BP resulting from exposure to 0.2 μg/m³ in the foundry is comparable to BP intake from smoking 5–7 packs per day.

Longitudinal studies

As with all cross-sectional studies, which provide a 'snapshot' at a particular point in time, this research cannot establish temporal or causal relationships between exposure, adduct formation and oncogene activation. For this reason, longitudinal studies in individuals whose exposure changes significantly over time are preferable in terms of establishing the relationship between exposure and biological markers such as macromolecular adducts. Few such studies have yet been conducted, but several 'natural experiments' have occurred such as studies of individuals in smoking cessation programmes, workers sampled before beginning employment in a coke-oven plant (Vähäkangas *et al.*, this volume), workers sampled after an interruption

Table 5. Levels of biological markers in subjects in a smoking cessation programme

	Stoppers			Non-stoppers		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
PAH–DNA (fmol/μg)	0.30 [0.095] (4)	—	0.19 [0.009] (4)	0.42 [0.07] (8)	—	0.21 [0.04] (8)
SCE (ave. met.)	10.94 [0.71] (8)	10.82 [0.35] (8)	9.65 [0.67†] (8)	12.57 [0.77] (10)	12.81 [0.55] (10)	12.23 [1.15] (10)
4-ABP–Hb (pg/g)	117.3 [30.85] (4)	74.8 [15.9] (4)	28.6 [7.73] (2)	140.8 [15.71] (6)	116.3 [26.78] (3)	—
Cotinine (ng/ml)	194 [77.55] (4)	1.75 [0.65] (4)	—	255.5 [31.6] (4)	163 [158.44] (2)	—

Sample 1 was taken at enrolment into the programme, sample 2 after 21 days and sample 3 at 3–6 months. Values are means [S.E.] with number of samples in parentheses.
†$p = 0.03$, Wilcoxon test
Source: Brenner *et al.* (unpublished)

in exposure (Haugen *et al.*, 1986) and chemotherapy patients from whom pretreatment and serial post-treatment samples have been obtained (Reed *et al.*, 1986; Fichtinger-Schepman *et al.*, 1987; Mustonen *et al.*, 1988; Perera *et al.*, 1989a).

Recently, we carried out a pilot study of smokers in a smoking cessation programme, obtaining repeat blood samples from 21 subjects upon enrolment into the programme, and then 21 days and 3–6 months after enrolment. Roughly half of the individuals quit smoking entirely within three weeks ('stoppers') and the other half either reduced smoking or continued to smoke at the same level ('non-stoppers'). As shown in Table 5, SCEs were significantly decreased in the stoppers (but not the non-stoppers) 3–6 months after quitting, while levels of 4-ABP–Hb and cotinine fell sharply within 21 days. However, PAH–DNA adducts were not significantly affected by smoking cessation, possibly because of their lack of specificity for cigarette smoke constituents and/or the short follow-up.

To build upon these results, we are beginning a more definitive study of similar subjects who will be followed for two years after quitting to evaluate persistence of DNA and haemoglobin adducts and oncogene protein products. We shall also evaluate several potential genetic/metabolic markers of susceptibility, such as aryl-hydrocarbon hydroxylase (AHH) activity and glutathione-*S*-transferase activity. This approach will allow detailed evaluation of the persistence of biological markers in human peripheral blood cells.

Another natural experiment has been provided by the annual vacation taken by Finnish foundry workers during the month of July. As shown in Table 4, white blood cell DNA from 12 individuals was assayed for PAH–DNA adducts after the workers returned from their four-week vacation. Nine of these subjects were sampled six weeks after their return to work. Four individuals were plant workers with low

exposure to PAH. The levels of adducts were significantly higher (0.92 ± 0.61 fmol/μg; $p = 0.004$, Wilcoxon test) in the post-work sample compared to the post-vacation sample (0.12 ± 0.14 fmol/μg), showing a clear biological effect of exposure.

Another longitudinal, serial sampling study has evaluated biological markers in patients treated with cisplatinum-based chemotherapy: cisplatinum (*cis*DDP) given in combination with Velban, mitomycin C, VP-16, adriamycin and/or 5-fluorouracil. Peripheral blood samples were collected just before and after the first, second and last cycles of chemotherapy and were assayed as shown in Table 6. In subjects with a baseline and at least two post-treatment samples, levels of SCE, plasma protein binding and haemoglobin-binding measured by atomic absorption spectrometry were significantly increased following treatment ($p < 0.01$). These results were consistent with prior studies of DNA and protein adducts and SCE in cisplatinum-treated patients (Reed *et al.*, 1986; R. Mustonen *et al.*, unpublished; Fichtinger-Schepman *et al.*, 1987). We have recently begun a follow-up study which will assess a full battery of markers (*cis*DDP–DNA and *cis*DDP–haemoglobin adducts, SCE and micronuclei, chromosomal aberrations, gene mutation (HPRT and GPA) and oncogene activation in peripheral blood cells and/or semen). This study will allow the effect of *cis*DDP to be separated from that of other genotoxicants administered, since adduct measurements are specific to *cis*DDP but the other markers will give an integrated measure of total genetic toxicity of chemotherapy. Correlation between *cis*DDP adducts and the non-specific markers will be particularly sought.

To summarize, the advantage of longitudinal serial sampling studies such as those described above is that the problem of interindividual variation is mitigated by having each individual serve as his or her own control. In addition, in the foundry workers study, all individuals were sampled on the same days so that possible seasonal variation in binding was controlled for. However, such research opportunities are rare, and although they address the temporal relationship between exposure and biomarkers, they leave unanswered the question of whether biomarkers are related to cancer risk.

Case–control studies

The first step in exploring the role of various biomarkers as risk factors for cancer has been taken in several modified case–control studies. These have a practical advantage over prospective studies in terms of feasibility and can provide circumstantial evidence of an association between biomarkers and cancer risk. However, as with cross-sectional studies, they cannot establish the causal sequence between adduct formation, gene mutation, oncogene activation and cancer. Moreover, the latency from exposure to cancer induction is usually years to decades and biological markers formed during exposure are likely to have been lost or diluted out by the time cancer is diagnosed. Unless exposure has been continuous and unchanged and metabolic processes have not altered over time, biological measurements made now are not relevant to present risk of cancer.

We have recently evaluated 81 cases with primary carcinoma of the lung and 67 controls for levels of PAH–DNA adducts and SCEs in order to determine whether,

Table 6. Levels of biomarkers in cisplatinum-treated patients by time of sample collection

	Baseline[a]	Post Cycle 1[b]	Pre Cycle 2[a]	Post Cycle 2[c]	Post Final Cycle[c]
Cumulative *cis* DPP dose (mg/m²)	0	99.23 ± 13.24	105.45 ± 28.41	197.00 ± 35.82	351.9 ± 60
SCE	10.80 ± 3.01 (12)	17.39 ± 6.52* (12)	17.45 ± 5.03* (12)	—	—
Plasma protein binding	0.47 ± 1.07 (10)	27.50 ± 9.34* (10)	2.37 ± 3.34 (2)	33.37 ± 11.07* (10)	33.49 ± 9.77* (10)
Haemoglobin binding	0.01 ± 0.04 (11)	2.08 ± 1.04* (11)		2.97 ± 1.25* (11)	2.89 ± 1.04 (4)

*$p < 0.01$ (Wilcoxon and *t*-tests)[d]

Values are means ± SD (standard deviation). Only subjects with measurements of baseline and two post-treatment samples are included; numbers of sample are given in parentheses.

[a] Samples drawn within 24 h before treatment

[b] SCE sample drawn 2 weeks after treatment; protein sample drawn 12–24 h post-treatment

[c] SCE and/or protein sample drawn 12–24 h post-treatment

[d] Analysis of difference between baseline and respective post-treatment values; both tests were two-tailed

Source: Perera *et al.* (1989a)

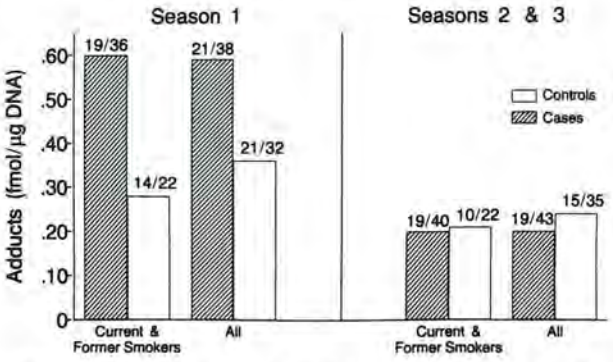

Figure 1. Polycyclic aromatic hydrocarbon–DNA adducts in white blood cells of lung cancer cases and controls (current smokers and former smokers; all subjects)
Values plotted are the means for positive samples; numbers above the bars represent the ratio of positive samples to all samples assayed. In Season 1 samples, the mean adduct level in lung cancer patients (former and current smokers combined) exceeded that in former and current smoker controls ($p = 0.02$, Wilcoxon test).

when exposure to PAHs and other mutagens or carcinogens was comparable, cases had higher levels of biomarkers than controls (Perera *et al.*, 1989b). This would suggest that the ability to efficiently activate and bind carcinogens may have been a risk factor in their disease. SCEs in lymphocytes did not differ between cases and controls. As in the smokers/non-smokers study, PAH–DNA adducts were not specific to cigarette smoking but apparently reflected the numerous background sources of PAHs such as diet and ambient air (Table 7). Among current smokers, white blood cell DNA from lung cancer cases had significantly higher levels of PAH–DNA adducts ($p = 0.01$, Wilcoxon test). As shown in Figure 1, this case–control difference was contributed by blood samples collected during the months July–October. This seasonal effect is consistent with the observation of seasonal variation in AHH activity in human lymphocytes (Paigen *et al.*, 1981). Genetically regulated metabolism of PAHs by the AHH microsomal enzyme system has been linked (in some but not all studies reported) to lung cancer risk (Kouri *et al.*, 1982; Kärki *et al.*, 1987).

As in the foundry workers study, we used western blotting techniques to screen sera of lung cancer cases and controls for peptide sequences of nine different proteins including *ras*, *fes* and *myc* (Brandt-Rauf *et al.*, 1989a). All 18 cases were positive for at least one oncogene protein, in contrast to two of the 20 healthy controls (Table 7). In 15 of these cases, all but one of which were non-squamous cell cancers, *ras* gene protein products were identified. Eleven of the 18 cases had activated *fes* compared to none of the controls. All 18 lung cancer cases were current or former cigarette smokers with impressive smoking histories (up to 105 pack years). These results are consistent with prior studies in that PAHs are known to activate the *ras* gene and a high prevalence of activated *ras* has been seen in non-small cell carcinomas and in

Table 7. DNA adducts and serum oncogene proteins in lung cancer patients

Patient	Age	Sex	Smoking history (pack-years)	Tumour type	Lymphocyte adducts (fmol/μg)	Oncogene protein								
						ras	*fes*	*myb*	*int*	*sis*	*myc*	*mos*	*src*	EGF
1	75	F	S(NA)	A	–	++	–	+	+	–	–	–	–	–
2	71	F	S(90)	A(S)	–	++	++	–	–	–	–	–	–	–
3	67	F	S(31)	A	–	++	–	–	–	–	–	–	–	–
4	42	M	S(20)	L	–	++	–	–	–	–	–	–	–	–
5	67	F	S(40)	A	–	++	–	–	–	–	–	–	–	–
6	82	M	S(55)	A	+(0.45)	++	–	–	–	–	–	–	–	–
7	49	M	S(63)	S	–	++	–	–	–	–	–	–	–	–
8	65	M	S(40)	L	+(0.16)	++	++	+	–	+	–	–	–	–
9	68	F	S(62)	A	–	++	++	–	–	–	–	–	–	–
10	62	M	S(61)	S	–	–	++	–	–	–	–	–	–	–
11	69	F	S(40	A	–	–	++	–	–	–	–	–	–	–
12	73	F	S(84)	S	–	–	–	–	+	–	–	–	–	–
13	67	F	S(105)	A	–	++	++	–	–	–	–	–	–	–
14	67	F	S(13)	L	–	++	++	–	–	–	–	–	–	–
15	60	F	S(20)	L	–	++	–	–	–	–	–	–	–	–
16	63	M	S(84)	A	–	++	++	–	–	–	–	–	–	–
17	69	M	S(50)	A	+(0.13)	++	++	–	–	–	–	–	–	–
18	62	M	S(30)	A	–	++	++	–	–	–	–	–	–	–
Controls														
19	58	M	NS			–								
20	25	M	NS			–								
21	30	M	ES			–								
22	31	M	CS			+ [All others negative]								
23	52	M	ES			+								
24	39	M	CS			–								
25	29	M	NS			–								
26	34	M	CS			–								
27	26	M	NS			–								
28	35	M	CS			–								
29	35	M	NS			–								
30	52	M	CS			–								
31	36	M	NS			–								
32	56	M	NS			–								
33	56	M	NS			–								
34	32	M	NS			–								
35	48	M	CS			–								
36	44	M	NS			–								

S, smoker; NS, non-smoker; CS, current smoker; ES, ex-smoker
++, strongly positive; +, positive; –, negative

A, adeno; AS, adenosquamous; L, large cell; S, squamous
Source: Brandt-Rauf *et al.* (1989a)

tumours from cigarette smokers. High levels of *fes* expression have also been detected in human lung tumours (reviewed in Brandt-Rauf *et al.*, 1989a).

PAH–DNA adducts and SCEs were measured in white blood cell DNA from these 18 patients. Three of these individuals had detectable levels of adducts, and all three were positive for the *ras* oncoprotein, but 11 of the *ras*-positive cases did not have detectable levels of adducts. Thus, there was no apparent correlation between adducts and oncogene activation, possibly because of the small number of subjects studied.

Nested case–control studies

As noted above, case–control studies have the serious limitation that if exposure or pharmacokinetic processes have altered between the time of cancer induction and diagnosis, current levels of macromolecular adduct levels may bear little relationship to cancer risk. Thus, the nested case–control is the optimal design for evaluating the relationship between biological markers and cancer risk, provided, of course, that the marker is stable in samples collected at the outset of a prospective study. In this approach, the cohort is followed and when cancer cases appear among the cohort, their stored biological samples are analysed and compared with those from one or more matched controls. This allows calculation of the relative risk, i.e., the measure of association between the incidence rates of cancer and the biomarker(s) being evaluated. Examples include a recent study of serum retinol and retinol binding protein and lung cancer (Friedman *et al.*, 1986) and an ongoing study of hepatitis B chronic carriers involving retrospective measurement of aflatoxin B_1 adducts on albumin and other markers once incident hepatocellular carcinoma cases are identified (Bosch & Muñoz, 1988).

Chemotherapy patients provide a valuable model for determining the predictive value of biomarkers to risk of cancer, since they may be at risk of second malignancy resulting from genotoxic chemotherapy. A multi-centre nested case–control study storing baseline and serial samples from cancer patients treated with cisplatinum-based chemotherapy would be both a practical and effective approach to marker validation.

Conclusions

Returning to the assessment of complex mixtures and multiple exposures, in light of the above discussion, our recommendations are as follows:

(1) Chemical-specific dosimeters (DNA and protein adducts) should be used in conjunction with non-specific markers such as SCE, micronuclei, gene mutation and oncogene activation which give an integrated estimate of the genotoxic/procarcinogenic effect of exposure.

(2) In order to separate the effect of specific constituents of the mixture, a step-wise evaluation can be carried out, as illustrated by the reverse pyramid in Figure 2. First, external exposure is characterized as completely as possible through ambient or personal monitoring and questionnaires. This provides an estimate of the

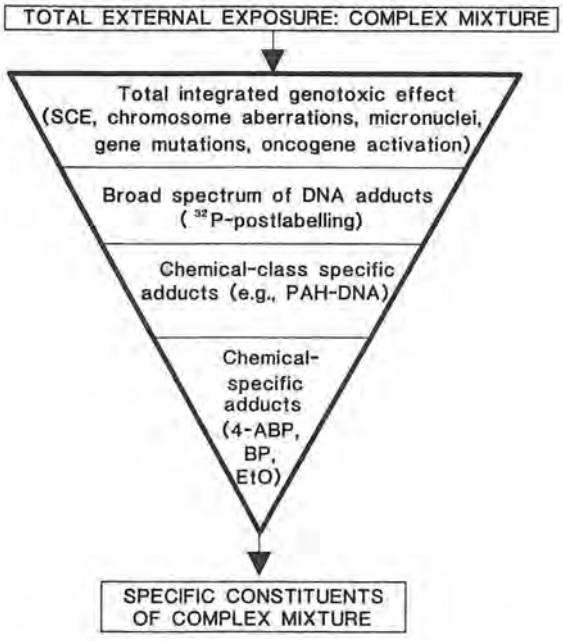

Figure 2. Assessment of the potential carcinogenic risk of complex mixtures

level and pattern of exposure both to the mixture and to its individual components. The next step is to analyse the relationship between integrated and chemical-specific exposure variables on the one hand and (*a*) total genotoxic/procarcinogenic effect, (*b*) the broad spectrum of DNA adducts by the postlabelling assay, (*c*) class-specific adducts (e.g., PAHs by immunoassay) and (*d*) individual chemical-specific adducts (e.g., 4-ABP–Hb or BP–DNA). Correlations between biological markers are also examined. The main question of interest is what proportion of the total genotoxic/procarcinogenic effect of exposure to complex mixtures is attributable to specific constituents in the mixture. Also, is there apparent interaction between individual constituents or do the effects appear to be additive? The answers to these questions may allow identification of the major pathogenic agents present in a chemical mixture.

(3) The most informative designs for identifying the relationship between exposure to complex mixtures and markers of biological dose and effect are serial sampling studies, which provide measurements at baseline and at several subsequent times after exposure has either ceased (e.g., smokers giving up the habit) or begun (e.g., newly appointed workers).

(4) To evaluate the role of complex mixtures in cancer risk, the nested case–control study is the optimal design. Suitable exposures for study include cigarette smoking, occupational exposures to fossil-fuel combustion products and combination chemotherapy.

In conclusion, the dramatic progress in the molecular biology of cancer during

the last five years has provided the impetus and the methods for a new, more powerful approach to cancer epidemiology and risk assessment. Provided the necessary groundwork is carried out, biological markers should become a valuable tool in cancer prevention.

Acknowledgements

We gratefully acknowledge the significant contribution of Dr I. B. Weinstein to these projects as well as that of Drs S. Tannenbaum and M. Bryant for analysis of aminobiphenyl adducts. We also thank Drs D. Warburton, M. Toor and H. K. Fischman for cytogenetic analyses, Dr B. M. Lee for contribution to DNA adduct assays and Dr M. Maclure for providing samples from subjects in the smoking cessation programme. This work was supported by Public Health Service Grants 5 R01CA-35809, 5 R01CA-39174, R01CA-47351-01 and grant P01CA-21111 from the Division of Extramural Activities, National Cancer Institute, by an NIEHS grant ES03881 and by grant 41387 from the American Cancer Society.

References

Autrup, H., Bradley, K. A., Shamsuddin, A. K. M., Wakhisi, J. & Wasunna, A. (1983) Detection of putative adduct with fluorescence characteristics identical to 2,3-dihydro-2-(7-guanyl)-3-hydroxyaflatoxin B1 in human urine collected in Murang'a District, Kenya. *Carcinogenesis*, **4**, 1193–1195

Balmain, A. & Pragnell, I. B. (1983) Mouse skin carcinomas induced in vivo by chemical carcinogens have a transforming Harvey-*ras* oncogene. *Nature*, **303**, 72–74

Bosch, F. X. & Muñoz, N. (1988) Prospects for epidemiological studies on hepatocellular cancer as a model for assessing viral and chemical interactions. In: Bartsch, H., Hemminki, K. & O'Neill, I. K., eds, *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention* (IARC Scientific Publications No. 89), Lyon, International Agency for Research on Cancer, pp. 427–438

Brandt-Rauf, P., Smith, S. & Perera, F. P. (1989a) Epidemiology and the environmental carcinogenesis of the lung. In: *Current Issues in Respiratory Public Health*, T. J. Witek and E. N. Schachter, eds, New York, J. B. Lippincott (in press)

Brandt-Rauf, P., Smith, S. & Perera, F. P., Niman, H. L., Yohannen, W., Hemminki, K. & Santella, R. M. (1989b) Serum oncogene proteins in foundry workers. *Am. J. Ind. Med.* (submitted for publication)

Bryant, M. S., Skipper, P. L., Tannenbaum, S. R. & MacLure, M. (1987) Hemoglobin adducts of 4-aminobiphenyl in smokers and nonsmokers. *Cancer Res.*, **47**, 612–618

Calleman, C. J., Ehrenberg, L., Jansson, B., Osterman-Golkar, S., Segerback, D., Svensson, K. & Wachtmeister, C. A. (1978) Monitoring and risk assessment by means of alkyl groups in hemoglobin in persons occupationally exposed to ethylene oxide. *J. Environ. Pathol. Toxicol.*, **2**, 427–442

Chacko, M. & Gupta, R. C. (1987) Evaluation of DNA damage in the oral mucosa of cigarette smokers and nonsmokers by ^{32}P–adduct assay. *Proc. Am. Assoc. Cancer Res.*, **28**, 101

Den Engelse, L., Terhaggen, P. M. A. B. & Begg, A. C. (1988) Cisplatin-DNA interaction products in sensitive and resistant cell lines and in buccal cells from cisplatin-treated cancer patients. *Proc. Am. Assoc. Cancer. Res.*, **29**, 339

Dunn, B. P. & Stich, H. F. (1986) ^{32}P postlabelling analysis of aromatic DNA adducts in human oral mucosal cells. *Carcinogenesis*, **7**, 1115–1120

Everson, R. E., Randerath, E., Santella, R. M., Cefalo, R. C., Avitts, T. A. & Randerath, K. (1986) Detection of smoking-related covalent DNA adducts in human placenta. *Science*, **231**, 54–57

Everson, R. E., Randerath, E., Santella, R. M., Avitts, T. A., Weinstein, I. B. & Randerath, K. (1988) Quantitative association between DNA damage in human placenta and maternal smoking and birth weight. *J. Natl Cancer Inst.*, **80**, 567–576

Farmer, P. B., Bailey, E., Gorf, S. M., Tornqvist, M., Osterman-Golkar, S., Kautiainen, A. & Lewis-Enright, D. P. (1986) Monitoring human exposure to ethylene oxide by the determination of hemoglobin adducts using gas chromatography-mass spectrometry. *Carcinogenesis*, **7**, 637–640

Fichtinger-Schepman, A. M. J., van Oosterom, A. T., Lohman, P. H. M. & Berends, F. (1987) Interindividual human variation in cisplatinum sensitivity, predictable in an in vitro assay? *Mutat. Res.*, **190**, 59–62

Friedman, G. D., Blaner, W. S., Goodman, D. S., Vogelman, J. H., Brind, J. L., Hoover, R., Fireman, B. H. & Orentreich, N. O. (1986) Serum retinol and retinol binding protein levels do not indicate subsequent lung cancer risk. *Am. J. Epidemiol.*, **123**, 781–789

Garner, R. C., Dvorackova, I. & Tursi, I. (1988) Immunoassay procedures to detect exposure to aflatoxin B1 and benzo[*a*]pyrene in animals and man at the DNA level. *Arch. Occup. Health*, **60**, 145–150

Groopman, J. D., Donahue, P. R., Zhu, J., Chen, J. & Wogan, G. N. (1985) Aflatoxin metabolism and nucleic acid adducts in urine by affinity chromatography. *Proc. Natl Acad. Sci. USA*, **82**, 6492–6496

Harris, C. C., Vähäkangas, K., Newman, N. J., Trivers, G. E., Shamsuddin, A., Sinopoli, N., Mann, D. L. & Wright, W. (1985) Detection of benzo[*a*]pyrene diol epoxide-DNA adducts in peripheral blood lymphocytes and antibodies to the adducts in serum from coke oven workers. *Proc. Natl Acad. Sci. USA*, **82**, 6672–6676

Harris, C. C. (1985) Future directions in the use of DNA adducts as internal dosimeters for monitoring human exposure of environmental mutagens and carcinogens. *Environ. Health Perspect.*, **62**, 185–191

Haugen, A., Becher, G., Vähäkangas, K., Benestad, C., Trivers, G. E., Newman, M. J. & Harris, C. C. (1986) Determination of polycyclic aromatic hydrocarbons in the urine, benzo[*a*]pyrene diol epoxide-DNA adducts in lymphocyte DNA, and antibodies to the adducts in sera from coke oven workers to measured amounts of polycyclic aromatic hydrocarbons in the work atmosphere. *Cancer Res.*, **46**, 4178–4183

IARC (1984) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 34, *Polynuclear Aromatic Compounds*, Part 3, *Industrial Exposures in Aluminium Production, Coal Gasification, Coke Production, and Iron and Steel Founding*, Lyon, International Agency for Research on Cancer

Kärki, N. T., Pokela, R., Nuutinen, L. & Pelkonen, O. (1987) Aryl hydrocarbon hydroxylase in lymphocytes and lung tissue from lung cancer patients and controls. *Int. J. Cancer*, **39**, 565–570

Kouri, R. E., McKinney, C. E., Slomiany, D. J., Snodgrass, D. R., Wray, N. P. & McLemore, T. L. (1982) Positive correlation between high aryl hydroxylase activity and primary lung cancer as analyzed in cryopreserved lymphocytes. *Cancer Res.*, **45**, 5030–5037

Liu, S. F., Rappaport, S. M., Pongracz, K. & Bodell, W. J. (1988) Detection of styrene oxide-DNA adducts in lymphocytes of a worker exposed to styrene. In: Bartsch, H., Hemminki, K. & O'Neill, I. K., eds, *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention* (IARC Scientific Publications No. 89), Lyon, International Agency for Research on Cancer, pp. 217–222

Marshall, C. J., Voosden, K. H. & Phillips, D. H. (1984) Activation of c-Ha-*ras*-1 proto-oncogene by in vitro modification with a chemical carcinogen, benzo[*a*]pyrene diol epoxide. *Nature*, **310**, 586–589

Miller, E. C. & Miller, J. A. (1981) Mechanisms of chemical carcinogenesis. *Cancer*, **47**, 1055–1064

Mustonen, R., Hemminki, K., Alhonen, A., Hietanen, P. & Kiilunen, M. (1988) In: Bartsch, H., Hemminki, K. & O'Neill, I. K., eds, *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention* (IARC Scientific Publications No. 89), Lyon, International Agency for Research on Cancer, pp. 329–332

Osterman-Golkar, S., Bailey, E., Farmer, P. B., Gorf, S. M. & Lamb, J. H. (1984) Monitoring exposure to propylene oxide through the determination of hemoglobin alkylation. *Scand. J. Work Environ. Health*, **10**, 99–102

Paigen, B., Ward, E., Reilly, A., Houten, L., Gurtoo, H. L., Minowada, J., Steenland, K., Havens, M. B. & Sartori, P. (1981) Seasonal variation of aryl hydrocarbon hydroxylase activity in human lymphocytes. *Cancer Res.*, **41**, 2757–2761

Perera, F. P. (1989) Molecular epidemiologic methods for assessing exposure and risk. In: Schottenfeld, D. & Fraumeni, J., eds, *Cancer Epidemiology and Prevention*, Oxford, Oxford University Press (in press)

Perera, F. P. & Weinstein, I. B. (1982) Molecular epidemiology and carcinogen-DNA adduct detection: New approaches to studies of human cancer causation. *J. Chron. Dis.*, **35**, 581–600

Perera, F. P., Santella, R. M., Brenner, D., Poirier, M. C., Munshi, A. A., Fischman, H. K. & Van Ryzin, J. (1987) DNA adducts, protein adducts and sister chromatid exchange in cigarette smokers and nonsmokers. *J. Natl Cancer Inst.*, **79**, 449–456

Perera, F. P., Hemminki, K., Young, T. L., Brenner, D., Kelly, G. & Santella, R. M. (1988a) Detection of polycyclic aromatic hydrocarbon-DNA adducts in white blood cells of foundry workers. *Cancer Res.*, **48**, 2288–2291

Perera, F. P., Santella, R. M., Brenner, D., Young, T. L. & Weinstein, I. B. (1988b) Application of biological markers to the study of lung cancer causation and prevention. In: Bartsch, H., Hemminki, K. & O'Neill, I. K., eds, *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention* (IARC Scientific Publications No. 89), Lyon, International Agency for Research on Cancer, pp. 451–459

Perera, F., Fischman, H. K., Hemminki, K., Brandt-Rauf, P., Niman, H. L., Smith, S., Tsai, W. Y. & Stoopler, M. (1989a) Protein binding, sister chromatid exchange and expression of oncogene proteins in patients treated with cisplatinum (cisDDP)-based chemotherapy. *Proc. Am. Assoc. Cancer Res.*, **30**, 283

Perera, F., Mayer, J., Jaretzki, A., Hearne, S., Young, T. L., Fischman, H. K., Grimes, M., Grantham, S., Tang, M. X., Tsai, W.-Y. & Santella, R. M. (1989b) Comparison of DNA adducts and sister chromatid exchange in lung cancer cases and controls. *Cancer Res.*, **49**, 4446–4451

Phillips, D. H., Hewer, A. & Grover, P. L. (1986) Aromatic DNA adducts in human bone marrow and peripheral blood leukocytes. *Carcinogenesis*, **7**, 2071–2075

Phillips, D. H., Hemminki, K., Alhonen, A., Hewer, A. & Grover, P. L. (1988a) Monitoring occupational exposure to carcinogens: detection by ^{32}P-postlabelling of aromatic DNA adducts in white blood cells from iron foundry workers. *Mutat. Res.*, **204**, 531–541

Phillips, D. H., Hewer, A., Martin, C. N., Garner, R. C. & King, M. M. (1988b) Correlation of DNA adducts levels in human lung with cigarette smoking. *Nature*, **336**, 790–792

Reddy, M. V. & Randerath, K. (1987) ^{32}P-postlabelling assay for carcinogen-DNA adducts: Nuclease P1-mediated enhancement of its sensitivity and applications. *Environ. Health Perspect.*, **76**, 41–47

Reed, E., Yuspa, S. H., Zwelling, L. A., Ozols, R. F. & Poirier, M. P. (1986) Quantitation of cis-diamminedichloroplatinum II (cis-platin)-DNA-intrastrand adducts in testicular and ovarian cancer patients receiving cisplatin chemotherapy. *J. Clin. Invest.*, **77**, 545–550

Santella, R. M., Yang, X. Y., De Leo, V. & Gasparro, F. P. (1988) Detection and quantification of 8-methoxypsoralen-DNA adducts. In: Bartsch, H., Hemminki, K. & O'Neill, I. K., eds, *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention* (IARC Scientific Publications No. 89), Lyon, International Agency for Research on Cancer, pp. 333–340

Shamsuddin, A. K. M., Sinopoli, N. T., Hemminki, K., Boesch, R. R. & Harris, C. C. (1985) Detection of benzo[*a*]pyrene-DNA adducts in human white blood cells. *Cancer Res.*, **45**, 66–68

Shuker, D. E. G. & Farmer, P. B. (1988) Urinary excretion of 3-methyladenine in humans as a marker of nucleic acid methylation. In: Bartsch, H., Hemminki, K. & O'Neill, I. K., eds, *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention* (IARC Scientific Publications No. 89), Lyon, International Agency for Research on Cancer, pp. 92–96

Törnqvist, M., Osterman-Golkar, S., Kautiainen, A., Jensen, A., Farmer, P. B. & Ehrenberg, L. (1986) Tissue doses of ethylene oxide in cigarette smokers determined from adduct levels in hemoglobin. *Carcinogenesis*, **7**, 1519–1521

Umbenhauer, D., Wild, C. P., Montesano, R., Saffhill, R., Boyle, J. M., Huh, N., Kirstein, U., Thomale, J., Rajewsky, M. F. & Lu, S. H. (1985) Methyldeoxyguanosine in oesophageal DNA among individuals at high risk of oesophageal cancer. *Int. J. Cancer*, **36**, 661–665

Vähäkangas, K., Trivers, G., Haugen, A. *et al.* (1985) Detection of benzo[*a*]pyrene diol-epoxide-DNA

adducts by synchronous fluorescence spectrophotometry and ultrasensitive enzymatic radioimmunoassay in coke oven workers. *J. Cell Biochem.*, Suppl. 9C, 1271

van Sittert, N. J., de Jong, G., Clare, M. G., Davies, R., Dean, B. J., Wren, L. J. & Wright, A. S. (1985) Cytogenetic, immunological, and hematological effects in workers in an ethylene oxide manufacturing plant. *Br. J. Ind. Med.*, **42,** 19–26

Weinstein, I. B., Gatoni-Celli, S., Kirschmeier, P., Lambert, M., Hsiao, W., Backer, J. & Jeffrey, A. (1984) Multistage carcinogenesis involves multiple genes and multiple mechanisms. *Cancer Cells*, 1. *The Transformed Phenotype*, Cold Spring Harbor Laboratory, NY, CSH Press, pp. 229–237

Weston, A., Willey, J. C., Manchester, D. K., Wilson, V. L., Brooks, B. R., Choi, J.-S., Poirier, M. C., Trivers, G. E., Newman, M. J., Mann, D. L. & Harris, C. C. (1988) In: Bartsch, H., Hemminki, K. & O'Neill, I. K., eds, *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention* (IARC Scientific Publications No. 89), Lyon, International Agency for Research on Cancer, pp. 181–189

Wild, C. P., Umbenhauer, D., Chapot, B. & Montesano, R. (1986) Monitoring of individual human exposure to aflatoxins (AF) and N-nitrosamines (NNO) by immunoassays. *J. Cell Biol.*, **30,** 171–179

Wogan, G. N. & Gorelick, N. J. (1985) Chemical and biochemical dosimetry of exposure to genotoxic chemicals. *Environ. Health Perspect.*, **62,** 5–18

Yuspa, S. H. & Poirier, M. C. (1989) Chemical carcinogenesis: From animal models to molecular models in one decade. *Adv. Cancer Res.* (in press)

WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

Complex Mixtures and Cancer Risk

Edited by
H. Vainio, M. Sorsa and A. J. McMichael

IARC Scientific Publications No. 104

International Agency for Research on Cancer
Lyon
1990

Published by the International Agency for Research on Cancer,
150 cours Albert Thomas, 69372 Lyon Cedex 08, France

Distributed by Oxford University Press, Walton Street, Oxford OX2 6DP, UK
Distributed in the USA by Oxford University Press, New York

ISBN 92 832 2104 4
ISSN 0300–5085

Printed in the United Kingdom