Appendix 1. DNA adducts

Table A1.1. DNA adducts NonSmokers

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Binkova 1998	Case--control	68 exposed workers, 56 controls (machine workers)	Blood (total peripheral white blood cells and lympho-cytes)	Occupa-tional (coke oven, Czech Republic, Slovakia)	DNA adducts	^{32}P-postlabelling		Smoking, genotypes, age, plasma levels of vita-mins A and E, BMI and diet	Each DNA sample was analysed in at least two independent postlabelling experiments with a variability of less than ±20%				Exposure to up to 62 μg/m^3 BP. Good correlation between total WBC and lym-phocites ($r = 0.591$, $p < 0.001$, $N = 124$). Adduct levels signifi-cantly elevated in workers compared with controls in both WBC and lympho-cytes. Smokers had significantly higher adduct levels in lym-phocytes. No effect of GSTM1 or NAT2 genotype, either sepa-rately or combined
Van Delft 1998	Case--control	19 low exposed (controls), 19 interme-diately exposed, 17 highly exposed workers	Blood, urine	Occupa-tional (carbon anode factory, The Nether-lands)	DNA adducts	^{32}P-postlabelling		Smoking	All samples were coded and analyzed in duplicate or triplicate				Exposure to up to 5 μg/m^3 BP. No difference in adduct levels between groups, although uri-nary 1-hydroxypyrene levels were significant-ly elevated in the inter-mediate and high exposure groups

Table A1.1. DNA adducts NonSmokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Kuljukka 1998	Cross--sectional	49 exposed workers, 10 controls coming from a near-by village	Peripheral white blood cells, urine, skin-wipe samples	Occupa-tional (cokery workers in oil shale processing plant, Estonia)	DNA adducts	^{32}P-postlabelling	No significant difference between adduct levels measured in the winter and in the fall season (data not shown)	Smoking					Exposure mean 10.4 μg/m^3 BP. Adducts in workers were not significantly different from controls ($p = 0.098$). Smokers had higher levels than non-smokers ($p = 0.002$). Adduct levels correlated with urinary 1-hydroxy--pyrene levels
Pan 1998	Cross--sectional	75 exposed workers, 24 controls (control plant, same city)	Blood (WBCs), urine	Occupa-tional (coke oven, China)	DNA adducts	^{32}P-postlabelling		Smoking, alcohol intake, food, genotype, PAH exposure at home (coal heating, coal cooking)	The measure-ments were performed in duplicate or triplicate for each sam-ple (data not shown, coeffi-cient of varia-ton not stated)				DNA adduct levels did not correlate with PAH exposure or uri-nary 1-hydroxypyrene. However, adduct levels correlated with 1-hydroxypyrene and alcohol consump-tion in workers with CYP1A1 Ile/Val or Val/Val polymor-phism. No influence of GSTM1
Whyatt 1998	Cross--sectional	70 mothers and new-born pairs	Umbilical cord blood + placental	Environ-mental (Poland)	DNA adducts	ELISA (BPDE-DNA antiserum)		Place of residence, smoking,					

		from Kraków and 90 pairs from Limanowa (vaginal deliveries only)	villus tissue					foods high in PAHs, use of coal stoves for residential heating and home/occupational exposure to PAHs and other oganics					
Arnould 1999	Cross -sectional	17 exposed workers, 10 controls	Blood (leukocytes), urine	Occupational (factory producing graphite electrodes from tar and lampblack)	DNA adducts	^{32}P-postlabelling ELISA (BPDE-I-DNA)			ELISA method: DNA-adduct detection were carried out twice in five dirfferent series, and negative control samples were used in each series of determination (data not shown). ^{32}P-postlabelling: DNA adduct detection were performed in triplicate (data not shown).				The DNA adducts due to B(a]P or its metabolites were measured by two methods, the ^{32}P-postlabelling and a competitive immunoassay. The detection limit was 0.15 fmol 50 mg 71 of DNA (postlabelling assay) and 10 fmol 50 mg 71 of DNA (immunoassay). In workers' group exposed to B(a]P, except two subjects (6 and 7), the total adduct levels ranged from 0.87 to 59.29 fmol 50 mg 71 of DNA by the ^{32}P-postlabelling method and from

Table A1.1. DNA adducts NonSmokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
Arnould 1999 – cont.													8.85 to 65.75 fmol 50 mg 71 of DNA by the immunoassay method. A regression between the two methods was shown: ELISA = 0.99 (Postlabelling) +13:65; F1;13 = 57:24; r^2 = 0:81; P < 0:001. The values obtained by the immunoassay method were signi-cantly higher than those obtained by the ^{32}P-postlabelling method (t14 = 5.40; P50.001). The lowest levels of adducts (by the two methods) were recorded in the less exposed non smoking workers (subjects 14, 15, 16 and 17) (Mann-Whitney U test, P = 0.010). But, we demonstrated no relationship

Arnould 1999 – cont.													between the quantity of adducts and the concentration of B(a)P.
Autrup 1999	Cross--sectional	107 healthy nonsmoking bus drivers (high and low exposed) 102 non-smoking mail carriers (medium and low exposed)	Blood (PMBCs) + + urine	Occupa-tional (bus drivers and mail carriers, Denmark)	DNA adducts	^{32}P-postlabelling		Smoking, eating habits, residence, activities invoving exposure to combus-tion products	The reported DNA-adducts is the average of at least two completely independent essays (less than twofold variation was observed in 85% of the analysed sam-ples) (data not shown)		A statisti-cally signi-ficant correlation was obser-ved between the content of total monocytes and the bulky car-cinogen-DNA ad-duct level (r =0.1564; p = 0.040; 192 cases), whereas no signifi-cant corre-lation was observed between adduct level and the content of B lym-phocytes		

Table A1.1. DNA adducts NonSmokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Autrup 1999 – cont.											(r = –0.097394; p = 0.20; 192 cases) or between T lymphocytes and the DNA adduct levels (r = = 0.0268; p = 0.71; 192 cases).		
Pavanello 1999	Cross--sectional	68 exposed workers (13 coke oven workers, 19 chimney sweeps, 36 allumini-um anode plant work-ers) and 26 controls	Blood (lym-phocite plus monocite fraction – LMF) +urine	Occupa-tional (coke oven, chimney sweeper and allu-minium anode, Italy)	DNA adducts	HPLC/fluores-cence of anti-BPDE tetrols		Genotype, type of occuppation	Calibration was carried out with calf-thy-mus DNA alone (back-ground) and spiked with 2, 4, 10, 20, 40, and 100 pg of anti-BPDE tetrol. The mini-mal correlation coeffcient was 0.98, and the mean coeff-		The risk of having high anti-BPDE-DNA adducts increases according to occupa-tional PAH exposure		The percentage of subjects whose adduct levels exceed-ed the 95 percentile control subject (46.7%) was signifi-cant ($x^{2\,test}$, $p < 0.01$).

									cient of variation for analyses repeated on different days was 16%				
Phillips 1999	Two inter-laboratory validation trials	1) Four standard samples (BaP--modified DNA; PhIP--modified DNA; ABP--modified DNA and unmodified calf thymus DNA which was used to prepare the modified DNA) + two samples (O^6- and N^7--methylated DNA and the unmodified calf thymus DNA) prepared by artice's authors 2) Five samples (two Ba-P-modi-	Lab generated samples	lab-generated exposures: Benzo(a]pyrene diolepoxide (3H-labelled); 4-amino--biphenyl (3H--labelled); PhIP (3H--labelled) Methylated (3H--labelled); BaP-DNA and ABP-DNA from mice's liver treated with triactonin	DNA adducts	^{32}P-postlabelling			In the second trial, analyses were carried out in duplicate on different days, results not reported	*			A recommended set of procedures has been developed for the detection and quantification of DNA adducts formed by PAHs, aromatic amines and methylating agents

Table A1.1. DNA adducts NonSmokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Phillips 1999 – cont.		fied DNA from mice's liver treated with trioctanoin; two ABP-modified DNA from mice's liver treated with trioctanoin; one unmodified DNA from liver of untreated mice) preapred by article's authors											
Schoket 1999	Cross--sectional	1) 172 aluminium plant workers 2) 48 garage mechanics from three garages in Budapest	Blood (lymphocites) + urine	Occupational (aluminium plant, garage mechanics and rubber vulcanising plant)	1) Aromatic DNA adducts + PAH-DNA adducts + 1-OH-PY 2) Aromatic DNA adducts + 1-OH-PY	1) ^{32}P-post-labelling + BPdG-DNA ELISA + HPLC/spectrofluometry 2) ^{32}P-post-labelling + HPLC/spectrofluometry		Smoking					The two methods (^{32}P-postlabelling and BPdG-DNA ELISA) provided similar qualitative evidence of genotoxic exposure in workers, however, there was a weak negative correlation between the two DNA

Schoket 1999 – cont.		3) 61 rubber vulcanising plant 4) 59 controls matched for age-range, smoking status and sex			+ micronucleus 3) Aromatic DNA adducts + Aromatic amine_DNA adducts + 1-OH-PY + somatic mutation	3) ^{32}P-postlabelling + G-C8-4-ABP DELFIA + HPLC/spectrofluometry GPA (form somatic mutations)							adduct biomarkers in individual pairs of data ($r = -0.232$; $p = 0.028$)
Viezzer 1999	Cross--sectional	98 exposed workers, 17 controls	Blood (mononuclear cells)	Occupational (coke oven from a steel plant, Italy)	DNA adducts	^{32}P-postlabelling		Genotype, smoking, age, sex, dietary habits, job category and years of exposure	All determinations were carried out in duplicate. The reproducibility of the method was checked by triplicate assays of each sample. Intra-assay variations were about 20%				GSTM1 *null* genotype increased DNA adduct levels in smoking workers with high PAH exposure. GSTT1 positive individuals had higher adduct levels than GSTT1 *null* ($p = 0.04$)
Palli 2000	Cross–sectional	309 subject (153 men) in the Epic Cohort	Peripheral white blood cells	Environmental + nutrition	DNA adducts	^{32}P-postlabelling (detection limit: 0.1 add/10^9 nucl)		Dietary and life-style habits	Approximately 20% of DNA samples verified with a second independent experiment: the results of the two analysis were in perfect agreement ($r = 0.98$)				

Table A1.1. DNA adducts NonSmokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Rojas 2000	Cross--sectional	89 exposed workers, 44 controls (power plant workers)	Blood (leukocytes)	Occupa-tional (coke oven, France)	DNA adducts	HPLC/fluores-cence of anti-BPDE adducts		Genotype, smoking status, age					PAH exposure had significant effects on adduct levels (p = 0.003, p = 0.006). Higher levels in individuals with certain CYP1A1 genotypes (*1/*2 or *2A/*2A) with *GSTM1 null* genotype
Rundle 2000	Hospital--based case-control	Tumor tissue samples from 100 breast tumor cases, normal tissue samples from 90 cases and tissue samples form 105 controls from 119 cases and 108 controls	Breast, blood	Environ-mental	DNA adducts	Immunohisto-chemical (with monoclonal antibody 5D11)		Age, age at menarche, parity, menopausal status, age at menopause, age at first birth, smoking	A single technician scored all the samples and a second technician re-scored a randomly selected subset of the samples: interclass correlation coefficient 0.80 (p = 0.0001) for tumor tissue, 0.72 (p = 0.0006)				The coefficient of variability was 20% for the positive control series and 29% for the negative control series. This represents the total variability due to intra-tumor differ-ences, lab variability and scoring variability and compares favor-ably with the coeffi-cient of variability of other methods for assessing adduct levels

Rundle 2000 – cont.									for benign tissue and 0.93 ($p < 0.0001$) for nontumor tissue				
Georgiadis 2001	Cross--sectional	117 non-smoking students living in Athens and 77 living in Halkida (rural area)	Blood	Environmental air pollution (PM2.5 - and associated PAH)	DNA adducts	^{32}P-postlabelling	**	Physical exercise, habitual consumption af barbecued or grilled meat	Some samples were analysed in two different experiments and the variability was found to be < 20%				A significant increase in th level of DNA adducts was observed in individuals who had been engaged in physical exercise indoord or outdoors during the previous 24 h
Van Delft 2001	Cross--sectional	35 exposed workers, 37 controls (unexposed workers of the same plant)	Blood	Occupational (coke oven, The Netherlands)	DNA adducts	^{32}P-postlabelling		Age, years at the coke oven, smoking habits, diet, genetic polymorphisms	Some samples analysed in two independent experiments: variability found to be < 20%				DNA adduct measure two methods: 1) DNA adducts TLC: PAH-DNA adducts per 108 nucleotides measured by the TLC method; 2) DNA adducts HPLC: PAH-DNA adducts in arbitrary units measured by the HPLC method. Workers did not have an increased level of adducts compared to controls, but smokers had higher levels than non-smokers ($p < 0.05$). No effect of GSTM1 or GSTP1 polymorphisms

Table A1.1. DNA adducts NonSmokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Ruchi-rawat 2002	Case--control	89 male, non smoking police officers (44 high and 45 low exposed to traffic pollutant)	Blood, urine	Occupa-tional, envi-ronmental (traffic) in Thailand	DNA adducts	^{32}P-postlabelling + (ELISA using an antibody (8E11) recognising bap tetrolsfro albumin adducts]	Low exposed (median ±SD): 0'-time: 1.2±1.0; week 1: 1.1±0.7; high exposed: 0'-time: 1.6±0.9; week 1: 1.8±1.3	Eating habits, residence, activities involving exposure to combustion products and petro-chemicals	The results are given as the mean of at least 2 independent assays. Coefficient of variation not stated				
Rundle 2002	Hospital--based case-control	Breast tissue from 104 cases (100 tumor samples, 90 non tumor samples) and from 105 controls	Breast, blood	Environ-mental	DNA adducts			Age, parity, age at first birth, age at menarche, ethnicity, breast-feed-ing status, family history of breats cancer, alcohol con-sumption	Interclass (2 lab techni-cians) correla-tion coefficient 0.82 ($p < 0.001$) in tumor tissue; 0.93 ($p < 0.001$) in non tumor tis-sue; and 0.74 ($p < 0.001$) in benign tissue				
Teixeira 2002	Cross--sectional	18 exposed workers, 21 controls	Blood	Occupa-tional (coke oven)	DNA adducts	^{32}P-postlabelling		Smoking status, alco-hol intake,	Each sample was analyzed at least in tripli-		Correlation between the level		DNA adduct levels were not significantly higher in workers

		(unexposed workers of the same plant)						medication use, X-ray exposure, occupational history and diet	cate in independent assays (data not shown)		of DNA adducts and cigarettes smoked was significant ($r = 0.70$; $p < 0.0005$) among controls, but no correlation was found among coke oven workers ($r = 0.12$; $p > 0.05$)		than in controls, although smokers had higher levels than non-smokers in both groups. Adduct levels in smokers were influenced by CYP1A1 Msp1 genotype but not by *GSTP1, GSTM1* and *GSTT1* polymorphisms
Farmer 2003	Cohort	200 men exposed to intense air pollution (policemen, bus drivers) and 150 matched controls in Prague (Czech Republic), Kosice (Slovak Republic) and Sofia (Bulgaria)	Blood, urine	Environmental air pollution	DNA adducts	^{32}P-postlabelling (fluorescence in situ hybridisation (fish) for chromosome aberration] (PCR-based genotype analysis for metabolic polymorphisms]		Smoking , dietary habits, genetic polymorphisms					

Table A1.1. DNA adducts NonSmokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Sørensen 2003	Cross--sectional over 1 year period	50 students living and studying in central Copenhagen studied 4 times over 1 year	Blood + urine	Environ-mental exposure to fine par-ticulate matter (personal pm 2.5 exposure; personal black smoke exposure; background pm 2.5 concentra-tion; and percentage of time exposed to ets)	DNA adducts	^{32}P-postlabelling	Median (q25-q75) PAH adducts (fmol/microg DNA) autumn 0.09 (0.06–0.14) winter 0.30 (0.20–0.48) spring 0.40 (0.26–0.60) summer 0.37 (0.23–0.59)	Season, gender, average outdoor temperature	Interessay coefficient of variation was <10% (an internal standard was used to correct for essay varia-bility and each sample was measured in at least two separate analysis). After measur-ing all the sam-ples, the meas-urement of 16 samples was repeated from different runs and seasons: the coefficient of variation between the separated analytical period was 12.6%				

Baran-czewski 2004	Cross--sectional	Liver form 15 healthy donors from the human cell culture centre (laurel, ma)	Liver	Environ-mental	DNA adducts	^{32}P-postlabelling and ^{32}P-HPLC analysis							
Ibañez 2005	Repeata-bility study	150 men and women form the Spanish epic cohort	Blood	Generic (reliability study of DNA adducts measure-ment)	DNA adducts	^{32}P-postlabelling			In 41 of the 150 subjects more DNA used to carry out a second independent measurement: interclass cor-relation coeffi-cient (ICC): 0.975 (95% CI 0.921–0.977)				
Peluso 2005	Nested case-control within epic cohort	4051 subjects (1,074 cases and 1,564	Blood	Environmen tal air pollu-tion	DNA adducts	^{32}P-postlabelling		Educational level, BMI, physical activity, fruits	Measurement of the adducts repeted in 27% of subjects		In general, there was not a clear-cut dose-		

Table A1.1. DNA adducts NonSmokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Peluso 2005 – cont.		controls); of them, 2410 blood samples (564 cases and 1,086 controls)						vegs meat and energy intake in addition to matching variables	(n = 311) r = 0.93 (p < 0.0001)		response relation-ship of lung cancer with increasing DNA adducts		

* Inter-lab CV (%) with trial method: BaP (nuclease P1) 65.41, (butanol) 69.77; PhIP (nuc. P1) 99.08, (butanol) 75.80; ABP (butanol) 93,41. Inter-lab CV (%) with investigator's own method: BaP (nuc. P1) 40.07, (butanol) 25.31; PhIP (nuc. P1) 105.42, (butanol) 45.83; ABP (butanol) 115.52.
Inter-lab CV (%) with trial method uncorrected and corrected on synthetic standards: Low mean BaP uncorrected 56.76, corrected 35.52; high mean BaP uncorrected 61.70, corrected 42.57; low mean ABP uncorrected 76.00, corrected 52.78; High mean ABP uncorrected 72.03, corrected 47.12.
** Winter vs. summer correlation of subjects ranking according to adducts level and exposure parametres:
Adduct A r = 0.043 (p = 0.659) in Athens, r = 0.060 (p = 0.735) in Halkida (minus campus); r = 0.305 (p = 0.059) in Halkida (campus).
Adduct B r = 0.058 (p = 0.549) in Athens, r = 0.245 (p = 0.162) in Halkida (minus campus); r = 0.451 (p = 0.004) in Halkida (campus).
Cotinine r = 0.322 (p = 0.001) in Athens, r = 0.324 (p = 0.062) in Halkida (minus campus); r = 0.298 (p = 0.065) in Halkida (campus).
PAH r = -0.051 (p = 0.633) in Athens, r = 0.094 (p = 0.623) in Halkida (minus campus); r = 0.351 (p = 0.053) in Halkida (campus).
B(a] P r = -0.038 (p = 0.705) in Athens, r = 0.117 (p = 0.522) in Halkida (minus campus); r = 0.304 (p = 0.072) in Halkida (campus).
Bper r = -0.025 (p = 0.802) in Athens, r = -0.008 (p = 0.967) in Halkida (minus campus); r = 0.433 (p = 0.008) in Halkida (campus).

Table A1.2. DNA adducts Smokers

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose response	Other source of variation	Notes
									Repeatability	Reproducibility			
Godschalk 1998	Cross--sectional + *in vitro* experiment	47 smokers, 62 non-smokers coming from 4 different studies: Maastricht I (blood from 8 healthy non smokers males), Maastricht II (blood from 5 male and 10 female non-smoking putatively unexposed), Lanken (blood and BAL from 8 male smokers with a suspected lung diasease), Amsterdam (26 males and 52 females	Blood (lymphocytes, monocytes, granulocytes) + bronchoalveolar lavage (BAL) cells	Lab generated exposure (treatment of blood cells with B(a)P for 18 h) Smoking	DNA adducts	^{32}P-postlabelling (detection limit < 1 add/10^9 nucl)			*In vitro*: Lymphocytes (from 5 healthy male volunteers) exposed to B(a)P in triplicate were analysed for DNA adducts using the NP1 enriched ^{32}P-postlabelling assay: the mean coefficient of variation was 18.2±2.9% (range: 2–40%, n = 5) for parallel experiments. *In vivo*: DNA samples analysed in 2 or 3 independent experiments to assess interessay variation	Differences in DNA isolation methods may partly be responsible for interlaboratory differences	A modest but significant linear relationship was observed between aromatic-DNA adduct levels in MNC detemined by NP1 mediated ^{32}P-postlabelling and tar exposure (excluding non--smokers: r = 0.31, P = 0.005), but not cigarette consumption (r = 0.18, P = 0.100) or pack-		Total adduct levels were highest in, respectively, BAL-cells (3.7±1.0, n = 5), peripheral blood monocytes (2.0±0.5, n = 8), lymphocytes (1.6±0.4, n = 8) and granulocytes (0.8±0.2, n = 8, P < 0.05) using NP1 enriched ^{32}P-postlabelling

Table A1.2. DNA adducts Smokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose response	Other source of variation	Notes
									Repeatability	Reproducibility			
Godschalk 1998 – cont.		healthy smoking volunteer)							±SE for lymphocytic DNA 19.1 ± 2.4% and for BAL-DNA 10.9 ± 2.5%		years (r = 0.14, P = 0.239).		
Schoket 1998	Cross--sectional	124 patients with lung malignancies and 26 with non-malignant lung conditions	Bronchial tissue	Environmental	DNA adducts	^{32}P-postlabelling		Genotype, smoking, type of lung disease	Values of DNA adducts levels were obtianed from 2 to 4 determinations in separate labelling assays. The assay variability was 25.4 ± 19.6% (mean ± SD)		Highly significant linear relationship between DNA adduct levels and logarithm of time of abstinence from smoking (n = 545, r = 5–0.471, P = 50.001). This function suggests an exponential elimination of DNA		A weak negative correlation was shown earlier between the the individual DNA adduct values determined by ^{32}P-postlabeling and immunoassay

											adducts from the bronchial tissue of former smokers with a fast early, and a slower later phase. In current smokers, there was no correla-tion between daily or cumulative cigarette dose and DNA adduct levels (r = 5–−0.0076 and 0.0072, respective-ly).		
Mollerup 1999	Cross--sectional	159 lung cancer patients: 122	Lung (normal tissue adjacent	Gender (on smok-ing-indu-ced	DNA adducts	^{32}P-postlabelling		Genotype, gene expression, age,					Adduct levels signifi-cantly higher in females than in males (p = 0.047 before

Table A1.2. DNA adducts Smokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Mollerup 1999 – cont.		smokers (29 females + 93 males), 37 non-smokers (13 female + 24 males)	to tumor tissue)	lung cancers)				smoking history					adjustment for pack-years, p = 0.0004 after adjustment). Lung expression of CYP1A1 (15 females, 12 males) was significantly higher in females (p = 0.016) and in both sexes correlation between CYP1A1 expression and adduct levels was significant (p = 0.009)25% of the variation in the level of DNA adducts could be explained by the variation in CYP1A1 expression
Ozawa 1999	Cross--sectional	143 lung patients (118 with lung malignancy + 25 undergoing pulmonary surgery for other lung contitions)	Lung (macroscopically normal bronchial tissue)	Genetic polimorphism (CYP2C9, GSTP1 and NQO1)	DNA adducts	^{32}P-postlabelling		Smoking	Assay variability 25.4 ± 19.6%				

Wiencke 1999	Case series	143 lung cancer patients undergoing surgery	Lung (surgically resected non-involved tissue) + blood (mononucleate cells)	Smoking (in particular age of initiation)	DNA adducts	^{32}P-postlabelling		Smoking history, occupation, demographic factors, genotype	Each sample was run at least 2 times on differnt days and the results were combined to abtain an average adduct level (coefficient of variation bfor repeated analyses of positive controls: 14%)				Smokers had significantly higher adduct levels than ex-smokers. Early age of commencing smoking associated with higher adduct levels in ex-smokers, but not in current smokers Spearman's correlation between DNA adducts level in blood mononucler cells and lung tissue: 0.77 ($p < 0.01$) The authors estimate that 60% of the variations in adduct measurements in lung tissue is explained by adduct measurements in the blood mononuclear cells
Cheng 2000	Case–control	73 patients with lung cancer and 33 controls (patients with non cancer lung diseases undergoing surgery)	Lung tissue (tumor-free areas surrunding the tumor for cancer cases)	Smoking	DNA adducts	^{32}P-postlabelling (detection limit: 1 add/10^9 nucl)		Age, gender, genetic polymorphisms (CYP1A1 and GSTM1)	Each sample was analysed in triplicate (data not shown)				Adduct levels were significantly higher in cases than in controls, but not higher in smokers than in non-smokers. Adduct levels not influenced by CYP1A1 Msp1 or GSTM1 genotypes.

Table A1.2. DNA adducts Smokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Piipari 2000	Cross--sectional	47 patients (31 smokers, 16 non-smokers) with suspected lung cancer or parenchymal lung disease undergoing Bronchoalveolar Lavage	Bronchoalveolar Lavage cells	Gene expression (CYP1A1, CYP1A2 and CYP1A3)	DNA adducts	^{32}P-postlabelling		Smoking, occupational history, exposure to asbestos and PAH					Adduct levels 3-fold higher in smokers than in non-smokers ($p < 0.001$) and correlated with cigarettes smoked daily. Smokers with high levels of CYP3A5 expression had higher adduct levels ($p < 0.002$)
Butkiewicz 2000	Cross--sectional	170 healthy men (120 smokers, 22 ex-smokers, 23 non-smokers)	Blood (mononuclear WBCs)	GSTP1 and GSTM1 polimorphism	DNA adducts	^{32}P-postlabelling	Mean number of DNA adducts by genotype higher in winter compared to summer for all polymorphism except GSTM1(null)/ /GSTP1-AG or -GG	Age, smoking status, PAH exposure					Adduct levels significantly higher in smokers compared with ex- and non-smokers. High adduct levels (upper quartile) significantly associated with CYP1A1 Val allele carriers among individuals who were GSTM1 null (n = 86)

Schoket 2001	Cross--sectional	1) 161 aluminium plant workers 2) 94 current cigarette smoking and short term ex-smokers patients undergoing lung surgery for various lung conditions, mostly lung cancer	1) Blood (lymphocites) 2) Normal bronchial tissue	1) PAH exposure 2) Cigarette smoking	DNA adducts	1) ^{32}P-postlabelling + ELISA 2) ^{32}P-postlabelling		Genotype (CYP1A1, CYP1B1, CYP2C9 and NQO1)					
Godschalk 2003	Cross--sectional (pilot study)	24 healthy smoker volunteers (14 male and 10 female) before and after quitting smoking	Blood (mononucleate cells: lymphocytes + monocytes)	Smoking cessation	DNA adducts	^{32}P-postlabelling (detection limit 0,1 add/10^8 nucl)	From $0.76 \pm 0,41$ to 0.44 ± 0.23 after 22 weeks of smoking cessation	Age, sex, BMI	Interassay variation < 20% (data not shown)				
Gyorffy 2004	Cross--sectional	85 lung cancer patients (47 smokers, 38 non-smokers)	Lung (lung tumour; distal lung tissue; bronchial tissue) + blood (peripheral	Smoking	DNA adducts	^{32}P-postlabelling (detection limit 0.3 add/10^8 nucl) immunoessay: BPDE-DNA CIA (detection limit		Histology, gender	^{32}P-postlabelling: two to four replicate analyses were performed with each human DNA sample, in separate				No significant difference in the DNA adduct levels in any tissue, regardless of the method, between males and females after stratification for histo-

Table A1.2. DNA adducts Smokers — cont.

Study	Study design	Sample size	Tissue /cells	Exposure	Biomarker	Laboratory technique	Variability of the test intra-individual	Confounders	Reliability of the test		Dose-response	Other source of variation	Notes
									Repeatability	Reproducibility			
Gyorffy 2004 – cont.			lympho-cytes)			1.1 ± 0.2 BPdG add/108 nucl)			assays. All the samples demonstrated detectable adduct levels. The assay variability was 28 ± 15%. BPDE-DNA CIA: 50% inhibition for the BPDE-DNA standard curve 2.69 ± 0.22 fmol BPdG/well (mean ± SEM, n = 17)				logical type or smoking category. An *in vitro*-modified BPDE-DNA standard was used in duplicate as external standard to reduce inter-assay variability In lung tumour weak correlation between values obtained by ^{32}P-postlabeling and BPDE-DNA immunoassay; in normal lung DNA samples no correlation
Bak 2006	Cohort	245 lung cancer cases (137 men and 108 women) and 255 controls (137 men and 118 women)	Blood (white blood cells)	Smoking	DNA adducts	^{32}P-postlabelling		Diet, smoking habits, education, environmental exposure, previous employment	Adduct levels were measured twice for each person, showing an average coefficient of variation of 6.2%. Seventeen replicate measurements				

		matched for sex, age ans smoking selected form the cohort							showed a coef-ficient of varia-tion for the 2 measurements of more than 20%, and for these, a third measurement was done to decrease the measurement uncertainty. For 2 of these 17, 1 of the 3 measure-ments was an extreme outlier, which was omitted from data. In the analyses, the mean of the 2 (or 3) meas-urements was used as the DNA adduct levels				