

Molecular dosimetry studies of smoking - induced carcinogenesis in target and surrogate tissues of humans

Citation for published version (APA):

Nia, A. B. (2001). *Molecular dosimetry studies of smoking - induced carcinogenesis in target and surrogate tissues of humans*. Universitaire Pers Maastricht.

Document status and date:

Published: 01/01/2001

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

CHAPTER 9

SUMMARY AND CONCLUSION

Chapter Nine

Summary and conclusion

A large body of evidence has shown that tobacco smoking is involved in the etiology of several human cancers (1,2). Tobacco smoke carcinogenicity is largely ascribed to its DNA-reactive constituents (3). These compounds comprise a wide range of chemicals with specific structures, which enable them to bind covalently to DNA and form DNA adducts (3). Formation of DNA adducts is an event of potential significance in carcinogenesis because it may give rise to chromosomal aberrations, DNA strand breaks, oncogene activation and tumor suppressor gene inactivation (4-6). By definition, DNA adducts are the biologically effective dose markers of exposure to carcinogens. That is that they not only represent a prior exposure to carcinogens but they also imply a risk for cancer (5). Of course, the implication of DNA adducts in cancer is not straightforward. The fact is that other processes such as cellular proliferation along with, or separate from DNA adduct formation may modulate carcinogenesis (5,7). Therefore, interpretation of DNA adduct data in relation to cancer ought to be done cautiously. Theoretically, dosimetry of DNA adducts should be performed within the organs where tumor arises [target organs]. Practically, however, most target organs for tobacco-associated cancers are only invasively accessible (8,9). Also, the commonly used non-target organs show inconsistent surrogacy for the target ones (10,11). The latter is mainly due to the incomparability of exposure patterns in surrogate *versus* target organs. In addition, different cell compositions with varying biotransformational and DNA repair capacities in target and surrogate organs may also be responsible for such inconsistency (12,13).

In the present thesis, we explored the carcinogenicity of tobacco smoke in humans by dosimetry of DNA adducts in various target and surrogate matrices. Focusing on three major classes of DNA adduct-inducing agents, polycyclic aromatic hydrocarbons (PAH), aromatic amines and reactive oxygen species (ROS), we studied the representative DNA adducts, benzo[a]pyrene diol epoxide (BPDE) -DNA adducts, 4-aminobiohenyl (4-ABP) -DNA adducts and 8-oxo-7,8-dihydroguanine (8-oxo-Gua), respectively (14-17).

In chapter 2, we examined the validity of DNA adduct analysis in induced sputum (IS), a non-invasive derivative from the lower airway (18). We detected significantly higher levels of smoke-related DNA adducts in IS of smokers as compared to non-smokers by both versions of the ³²P-postlabeling assay, the nuclease P1 digestion and the butanol extraction methods. The similarity and correlation between the levels of adducts quantified by different enhancement methods confirm the previous findings by others indicating the PAH-derived nature of adducts in the lower respiratory tract (19-21). Technically, the NP1 digestion method degrades the C8-guanine-adducted nucleotides of aromatic amines and substantially enhances the N²-guanine adducts, whereas the butanol extraction method enriches both types of adducts (22). Furthermore, the dose-dependency of smoke-related DNA adducts in IS suggests that this matrix can be used for molecular dosimetry of inhalatory carcinogens. Also, the comparability of our results to those obtained in bronchoalveolar lavage (BAL) cells, an already validated but invasively accessible matrix (23-25), implies that sputum induction may replace the BAL method for sampling airway.

In chapter 3, we validated DNA adduct dosimetry in IS and further, compared it with dosimetry of DNA adducts in peripheral blood lymphocytes (PBL) (26). First and foremost, DNA adduct analysis in IS produced similar results to what we had previously reported (18). Also, repeated measurement of DNA adducts in IS and PBL showed a consistency in the level of adducts in both matrices [over a three-week period]. Comparatively, adduct analysis in IS was more explicit than that in PBL both qualitatively and quantitatively. Moreover, there were some indications of the persistency of DNA adducts in PBL. For example, DNA adduct levels in PBL were dependent on the cumulative dose of exposure to tobacco smoke [pack years]. In addition, the levels of adducts in PBL did not drop as drastic as those in IS after smoking cessation. Altogether, it appears that dosimetry of DNA adducts in IS is a choice of preference for studying tobacco smoke carcinogenicity.

In chapter 4, we separately quantified aromatic amine- and PAH-DNA adducts in IS by immunohistochemistry of 4-ABP- and BPDE-DNA adducts, respectively (27). We found that the levels of both types of adducts were dose-dependently related to the current smoking intensity; however, the results were more pronounced for 4-ABP-DNA adducts. On the one hand, this shows the specificity of immunohistochemistry of 4-ABP-DNA adducts. On the other hand, it suggests that ubiquitous confounding exposure to PAH (28) may impact upon immunohistochemistry of BPDE-DNA adducts. Undoubtedly, the high levels of BPDE-DNA adducts in some of the non-smokers and the wider range of BPDE-DNA adducts as compared to 4-ABP-DNA adducts in non-smokers reinforce this view.

In chapter 5, we used DNA adduct dosimetry in IS and PBL together with ambient air monitoring for assessing non-smokers' exposure to environmental tobacco smoke (ETS). Our air monitoring data showed that spending an average period of time in a smoky pub results in relatively high exposure to ETS. Accordingly, we found reasonable enhancement in smoke-related DNA adducts in IS but not in PBL after the pub visit. Of most significance was the formation of BPDE-DNA adducts in IS of a few individuals at post-exposure time. Noteworthy, this adduct is formed at the mutational hotspots of lung cancer (6,29) for which ETS exposure is a known risk factor (30-33). Taken together, the results of this pilot study indicate that DNA adduct analysis in IS might potentially be used as an integral approach to assess ETS exposure as well as to study ETS-related cancers.

In chapter 6, we explored the relevance of markers of oxidative DNA damage/repair as well as antioxidative defense mechanisms for studying tobacco smoke carcinogenicity (34). Although it is a known fact that smoking induces oxidative stress, we found a down regulation of ROS-induced DNA damage (17) in smokers. We hypothesized that this phenomenon may occur as a result of adaptation of antioxidative defense and/or DNA repair systems in smokers. However, quantification of the antioxidative capacity of plasma and genotyping of a relevant antioxidant enzyme, glutathione *S*-transferase M1 (*GSTM1*) did not support this hypothesis. Also, measurement of the overall DNA repair activity and genotyping of a specific DNA repair enzyme, human 8-hydroxyl-2'-deoxyguanosine (8-OH-dG) - glycosylase/apurinic lyase (*hOGGI*) were not supportive of this hypothesis. Given the controversial results of other studies (35-41), we may consider that the herein-quantified markers are not specific and sensitive to show the subtle effects of smoking. For example, the low prevalence of *hOGGI* polymorphism may easily mask the impact of this genotype on DNA repair pathway in small-scale studies. It is worthy mentioning that in our study most

evaluated pathways were highly influenced by host co-factors. For instance, our multiple regression analysis revealed that the non-significant up regulation of plasma antioxidants scavenging capacity in smokers was mainly gender-related. Accordingly, adjustment of data for gender bridged the gap between smokers' and non-smokers' antioxidative capacity. Moreover, males who are under greater oxidative burden due to their higher metabolic rates (42), had lower level of oxidative DNA damage, which was explained by their elevated plasma antioxidants scavenging capacity. Altogether, it seems that the current markers of oxidative DNA damage/repair and antioxidative defense mechanisms need further validation before they can be used for studying tobacco smoke carcinogenicity in humans.

In chapter 7, we investigated the predictive value of DNA adducts for exposure to tobacco smoke and risk for oral cancer (43). Immunohistochemistry of PAH-DNA adducts in two different regions of the oral cavity with varying cancer susceptibility profiles (44-46) showed a dose-dependency of DNA adducts with regard to current smoking intensity. However, PAH-DNA adduct levels *in situ* did not correspond with the cancer proneness of the respective subsite. In fact, mouth floor cells, which are highly cancer susceptible had lower level of DNA adducts compared to buccal mucosa cells. Apparently, DNA adduct formation in the oral cavity is a consequence of exposure to tobacco smoke rather than a pre-requisite for developing oral cancer. Thus, dosimetry of DNA adducts in the oral cells can safely mirror the exposure to tobacco smoke. Yet, the complexity of the multi-stage process of carcinogenesis (5) makes it unpredictable by DNA adduct dosimetry *per se*.

In chapter 8, we quantified various smoke-associated markers to verify the efficacy of chemopreventive agent *N*-acetyl-L-cysteine (NAC) in humans. We assessed the effects of NAC on different biological endpoints because NAC has been shown to exert its effects through a variety of coordinated mechanisms (47-50). Accordingly, we found that NAC could modulate certain markers within specific matrices. For example, NAC significantly inhibited the formation of DNA adducts in the BAL cells but not in the oral mucosa or PBL. Also, we observed a dual effects of NAC [efficacy/inefficacy] within the individual matrices. For instance, the selectivity of NAC in inhibiting DNA adducts, boosting antioxidative capacity and preventing cytogenetic damages [micronuclei frequency] were independent of each other. Taken together, our results reaffirm the previous experimental data, which have shown a tissue specificity for uptake and efficacy of NAC (51-62). At the same time, they reiterate the importance of a multi-biomarker approach to study the underlying mechanisms of carcinogenesis.

Summarizing the herein-presented data and those from the literature, we may consider that dosimetry of PAH- and aromatic amine-DNA adducts in target and relevant surrogate matrices eg., induced sputum is a valuable means to study tobacco smoke carcinogenicity in humans. However, the relevance of ROS-induced DNA adducts and other oxidative stress-associated markers for such study remain to be seen. To better understand the smoking-induced carcinogenesis in humans and the roles that are played by chemical carcinogens in it, future large-scale research is needed. Such investigations should be conducted in a multi-disciplinary way to elucidate the impact of specific DNA adducts in target and validated surrogate matrices, along with genotyping/phenotyping of biotransformational and DNA repair enzymes.

REFERENCE

1. Pisani, P., Parkin D.M., Bray F. and Ferlay J. (1999) Estimates of the worldwide mortality from 25 cancers in 1990. *Int J Cancer*, **83**, 18-29.
2. Ferlay, J., Parkin D.M. and Pisani P. (1998) GLOBOCAN. In International Agency for Research on Cancer. Cancer Base (ed.), *Cancer incidence and mortality worldwide (CD-rom)*. Vol. 3. International Agency for Research on Cancer, Lyon (France).
3. Hecht, S.S. (1996) Carcinogenesis due to tobacco: molecular mechanisms. In Bertino, J.R. (ed.), *Encyclopedia of cancer*. Academic Press, San Diego (CA), pp. 220-232.
4. Ross, J.A., Nelson G.B., Wilson K.H., Rabinowitz J.R., Galati A., Stoner G.D., Nesnow S. and Mass M.J. (1995) Adenomas induced by polycyclic aromatic hydrocarbons in strain A/J mouse lung correlate with time-integrated DNA adduct levels. *Cancer Res*, **55**, 1034-1044.
5. Hemminki, K. (1993) DNA adducts, mutations and cancer. *Carcinogenesis*, **14**, 2007-2012.
6. Denissenko, M.F., Pao A., Tang M. and Pfeifer G.P. (1996) Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in *P53*. *Science*, **274**, 430-432.
7. Goldring, J. and Lucier G. (1990) Protein and DNA adducts. In Hulka, B.S., Wilcosky, T. and Griffith, J. (eds), *Biological markers in epidemiology*. Vol. 1. Oxford University Press, New York, pp. 78-104.
8. Wilcosky, T.C. and Griffith J.D. (1990) Application of biological markers. In Hulka, B.S., Wilcosky, T.C. and Griffith, J.D. (eds), *Biological markers in epidemiology*. Vol. 1. Oxford University Press, New York, pp. 16-27.
9. Wilcosky, T.C. (1990) Criteria for selecting and evaluating markers. In Hulka, B.S., Wilcosky, T.C. and Griffith, J.D. (eds), *Biological markers in epidemiology*. Oxford University Press, New York, pp. 28-55.
10. Poirier, M.C. and Weston A. (1996) Human DNA adduct measurements: state of the art. *Environ Health Perspect*, **104 Suppl 5**, 883-893.
11. Phillips, D.H. (1996) DNA adducts in human tissues: biomarkers of exposure to carcinogens in tobacco smoke. *Environ Health Perspect*, **104 Suppl 3**, 453-458.
12. Butkiewicz, D., Grzybowska E., Hemminki K., Ovrebø S., Haugen A., Motykiewicz G. and Chorazy M. (1998) Modulation of DNA adduct levels in human mononuclear white blood cells and granulocytes by *CYP1A1*, *CYP2D6* and *GSTM1* genetic polymorphisms. *Mutat Res*, **415**, 97-108.
13. Knudsen, L.E., Ryder L.P. and Wasserman K. (1992) Induction of DNA repair synthesis in human monocytes/B-lymphocytes compared with T-lymphocytes after exposure to *N*-acetoxy-*N*-acetylaminofluorene and dimethylsulfate *in vitro*. *Carcinogenesis*, **13**, 1285-1287.
14. Hoffmann, D. and Hoffmann I. (1997) The changing cigarette, 1950-1995. *J Toxicol Environ Health*, **50**, 307-364.
15. International Agency for Research on Cancer (1986) Tobacco smoking, *Monographs on the evaluation of the carcinogenic risk of chemicals to humans*. Vol. 38. International Agency for Research on Cancer, Lyon (France), pp. 37-375.
16. Beland, F.A. and Kadlubar F.F. (1990) Metabolic activation and DNA adducts of aromatic amines and nitroaromatic hydrocarbons. In Cooper, C.S. and Grover, P.L. (eds), *Chemical carcinogenesis and mutagenesis I*. Vol. 94/I. Springer-Verlag, Berlin-Heidelberg, pp. 297-325.
17. Cheng, K.C., Cahill D.S., Kasai H., Nishimura S. and Loeb L.A. (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G → T and A → C substitutions. *J Biol Chem*, **267**, 166-172.

18. Nia, A.B., Maas L.M., Van Breda S.G., Curfs D.M., Kleinjans J.C., Wouters E.F. and Van Schooten F.J. (2000) Applicability of induced sputum for molecular dosimetry of exposure to inhalatory carcinogens: ³²P-postlabeling of lipophilic DNA adducts in smokers and nonsmokers. *Cancer Epidemiol Biomarkers Prev*, **9**, 367-372.
19. Van Schooten, F.J., Hillebrand M.J., Van Leeuwen F.E., Lutgerink J.T., Van Zandwijk N., Jansen H.M. and Kriek E. (1990) Polycyclic aromatic hydrocarbon-DNA adducts in lung tissue from lung cancer patients. *Carcinogenesis*, **11**, 1677-1681.
20. Weston, A. and Bowman E.D. (1991) Fluorescence detection of benzo[a]pyrene-DNA adducts in human lung. *Carcinogenesis*, **12**, 1445-1449.
21. Alexandrov, K., Rojas M., Geneste O., Castegnaro M., Camus A.M., Petruzzelli S., Giuntini C. and Bartsch H. (1992) An improved fluorometric assay for dosimetry of benzo[a]pyrene diol-epoxide-DNA adducts in smokers' lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. *Cancer Res*, **52**, 6248-6253.
22. Gupta, R.C. (1993) ³²P-postlabelling analysis of bulky aromatic adducts. In Phillips, D.H., Castegnaro, M. and Bartsch, H. (eds), *Postlabeling methods for detection of DNA adducts*. International Agency for Research on Cancer, Lyon.
23. Izzotti, A., Rossi G.A., Bagnasco M. and De Flora S. (1991) Benzo[a]pyrene diolepoxide-DNA adducts in alveolar macrophages of smokers. *Carcinogenesis*, **12**, 1281-1285.
24. Van Schooten, F.J., Godschalk R.W., Breedijk A., Maas L.M., Kriek E., Sakai H., Wigbout G., Baas P., Van't Veer L. and Van Zandwijk N. (1997) ³²P-postlabelling of aromatic DNA adducts in white blood cells and alveolar macrophages of smokers: saturation at high exposures. *Mutat Res*, **378**, 65-75.
25. De Flora, S., Izzotti A., D'Agostini F., Rossi G.A. and Balansky R.M. (1993) Pulmonary alveolar macrophages in molecular epidemiology and chemoprevention of cancer. *Environ Health Perspect*, **99**, 249-252.
26. Nia, A.B., Maas L.M., Brouwer E.M., Kleinjans J.C. and Van Schooten F.J. (2000) Comparison between smoking-related DNA adduct analysis in induced sputum and peripheral blood lymphocytes. *Carcinogenesis*, **21**, 1335-1340.
27. Nia, A.B., Van Straaten H.W., Kleinjans J.C. and Van Schooten F.J. (2000) Immunoperoxidase detection of 4-aminobiphenyl- and polycyclic aromatic hydrocarbons-DNA adducts in induced sputum of smokers and non-smokers. *Mutat Res*, **468**, 125-135.
28. International Agency for Research on Cancer (1985) Monographs on the evaluation of the carcinogenic risk of chemicals to humans: polynuclear aromatic compounds. Vol. part 4. International Agency for Research on Cancer, Lyon (France).
29. Smith, L.E., Denissenko M.F., Bennett W.P., Li H., Amin S., Tang M. and Pfeifer G.P. (2000) Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. *J Natl Cancer Inst*, **92**, 803-811.
30. US Environmental Protection Agency (1992) Respiratory health effects of passive smoking: lung cancer and other disorders. US Environmental Protection Agency, Office of Health and Environmental Assessment, Office of Research and Development, Washington D.C., EPA/600/6-90/006F.
31. Spitzer, W.O., Lawrence V., Dales R., Hill G., Archer M.C., Clark P., Abenheim L., Hardy J., Sampalis J., Pinfold S.P. and et al. (1990) Links between passive smoking and disease: a best-evidence synthesis. A report of the Working Group on Passive Smoking. *Clin Invest Med*, **13**, 17-42.
32. Tredaniel, J., Boffetta P., Saracci R. and Hirsch A. (1994) Exposure to environmental tobacco smoke and risk of lung cancer: the epidemiological evidence. *Eur Respir J*, **7**, 1877-1888.

33. Leonard, C.T. and Sachs D.P.L. (1999) Environmental tobacco smoke and lung cancer incidence. *Curr Opin Pul Med*, **5**, 189-193.
34. Nia, A.B., Van Schooten F.J., Schilderman P.A.E.L., De Kok T.M.C.M., Haenen G.R., Van Herwijnen M.H.M., Van Aagen E., Pachon D. and Kleinjans J.C. (2001) A multi-biomarker approach to study smoking-induced oxidative DNA damage and repair and antioxidative defense mechanisms. *Carcinogenesis*, **22**, 101-107
35. Loft, S., Vistisen K., Ewertz M., Tjonneland A., Overvad K. and Poulsen H.E. (1992) Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*, **13**, 2241-2247.
36. Loft, S., Poulsen H.E., Vistisen K. and Knudsen L.E. (1999) Increased urinary excretion of 8-oxo-2'-deoxyguanosine, a biomarker of oxidative DNA damage, in urban bus drivers. *Mutat Res*, **441**, 11-19.
37. Loft, S., Fischer-Nielsen A., Jeding I.B., Vistisen K. and Poulsen H.E. (1993) 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage. *J Toxicol Environ Health*, **40**, 391-404.
38. Yin, B., Whyatt R.M., Perera F.P., Randall M.C., Cooper T.B. and Santella R.M. (1995) Determination of 8-hydroxydeoxyguanosine by an immunoaffinity chromatography-monoclonal antibody-based ELISA. *Free Radic Biol Med*, **18**, 1023-1032.
39. Hardie, L.J., Briggs J.A., Davidson L.A., Allan J.M., King R.F., Williams G.I. and Wild C.P. (2000) The effect of *hOGG1* and glutathione peroxidase I genotypes and 3p chromosomal loss on 8-hydroxydeoxyguanosine levels in lung cancer. *Carcinogenesis*, **21**, 167-172.
40. Shinmura, K., Kohno T., Kasai H., Koda K., Sugimura H. and Yokota J. (1998) Infrequent mutations of the *hOGG1* gene, that is involved in the excision of 8-hydroxyguanine in damaged DNA, in human gastric cancer. *Jpn J Cancer Res*, **89**, 825-828.
41. Sugimura, H., Kohno T., Wakai K., Nagura K., Genka K., Igarashi H., Morris B.J., Baba S., Ohno Y., Gao C., Li Z., Wang J., Takezaki T., Tajima K., Varga T., Sawaguchi T., Lum J.K., Martinson J.J., Tsugane S., Iwama S., Shinmura K. and Yokota J. (1999) *hOGG1* Ser326Cys polymorphism and lung cancer susceptibility. *Cancer Epidemiol Biomarkers Prev*, **8**, 669-674.
42. Meijer, G.A., Westerterp K.R., Saris W.H. and ten Hoor F. (1992) Sleeping metabolic rate in relation to body composition and the menstrual cycle. *Am J Clin Nutr*, **55**, 637-640.
43. Nia, A.B., Van Straaten H.W., Godschalk R.W., Van Zandwijk N., Balm A.J., Kleinjans J.C. and Van Schooten F.J. (2000) Immunoperoxidase detection of polycyclic aromatic hydrocarbon-DNA adducts in mouth floor and buccal mucosa cells of smokers and nonsmokers. *Environ Mol Mutagen*, **36**, 127-133.
44. Hicks, W.L., Jr., Loree T.R., Garcia R.I., Maamoun S., Marshall D., Orner J.B., Bakamjian V.Y. and Shedd D.P. (1997) Squamous cell carcinoma of the floor of mouth: a 20-year review. *Head Neck*, **19**, 400-405.
45. Negri, E., La Vecchia C., Levi F., Franceschi S., Serra-Majem L. and Boyle P. (1996) Comparative descriptive epidemiology of oral and oesophageal cancers in Europe. *Eur J Cancer Prev*, **5**, 267-279.
46. Swango, P.A. (1996) Cancers of the oral cavity and pharynx in the United States: an epidemiologic overview. *J Public Health Dent*, **56**, 309-318.
47. De Flora, S., Balansky R., Benniselli C., Camoirano A., D'Agostini F., Izzotti A. and Cesarone C. (1995) Mechanisms of anticarcinogenesis: the example of *N*-acetylcysteine. In Ioannides, C. and Lewis, D.F.V. (eds), *Drugs, Diet and Disease*. Vol. 1. Mechanistic approaches to cancer. Horwood Ellis, Hemel Hempstead, pp. 151-203.

48. De Flora, S. and Ramel C. (1988) Mechanisms of inhibitors of mutagenesis and carcinogenesis. Classification and overview. *Mutat Res*, **202**, 285-306.
49. De Flora, S., Bagnasco M. and Zanacchi P. (1992) Classification and mechanism of action of chemopreventive compounds. In De Palo, G., Sporn, M. and Veronesi, U. (eds), *Progress and Perspectives in Chemoprevention of Cancer*. Raven Press, New York, pp. 1-11.
50. De Flora, S., Izzotti A. and Bennicelli C. (1993) Mechanisms of antimutagenesis and anticarcinogenesis. Role in primary prevention. In Bronzetti, G., Hayatsu, H., De Flora, S., Waters, M.D. and Shankel, D.M. (eds), *Antimutagenesis and Anticarcinogenesis Mechanisms III*. Plenum Press, New York, pp. 1-16.
51. De Flora, S., Cesarone C.F., Balansky R.M., Albini A., D'Agostini F., Bennicelli C., Bagnasco M., Camoirano A., Scatolini L., Rovida A. and et al. (1995) Chemopreventive properties and mechanisms of *N*-Acetylcysteine. The experimental background. *J Cell Biochem Suppl*, **22**, 33-41.
52. De Flora, S., Astengo M., Serra D. and Bennicelli C. (1986) Inhibition of urethane-induced lung tumors in mice by dietary *N*-acetylcysteine. *Cancer Lett*, **32**, 235-241.
53. Rogers, D.F. and Jeffery P.K. (1986) Inhibition by oral *N*-acetylcysteine of cigarette smoke-induced "bronchitis" in the rat. *Exp Lung Res*, **10**, 267-283.
54. Wilpart, M., Speder A. and Roberfroid M. (1986) Anti-initiation activity of *N*-acetylcysteine in experimental colonic carcinogenesis. *Cancer Lett*, **31**, 319-324.
55. De Flora, S., D'Agostini F., Izzotti A. and Balansky R. (1991) Prevention by *N*-acetylcysteine of benzo[a]pyrene clastogenicity and DNA adducts in rats. *Mutat Res*, **250**, 87-93.
56. Pereira, M.A. and Khoury M.D. (1991) Prevention by chemopreventive agents of azoxymethane-induced foci of aberrant crypts in rat colon. *Cancer Lett*, **61**, 27-33.
57. Bagnasco, M., Bennicelli C., Camoirano A., Balansky R.M. and De Flora S. (1992) Metabolic alterations produced by cigarette smoke in rat lung and liver, and their modulation by oral *N*-acetylcysteine. *Mutagenesis*, **7**, 295-301.
58. Balansky, R.B., D'Agostini F., Zanacchi P. and De Flora S. (1992) Protection by *N*-acetylcysteine of the histopathological and cytogenetical damage produced by exposure of rats to cigarette smoke. *Cancer Lett*, **64**, 123-131.
59. Izzotti, A., Balansky R.M., Coscia N., Scatolini L., D'Agostini F. and De Flora S. (1992) Chemoprevention of smoke-related DNA adduct formation in rat lung and heart. *Carcinogenesis*, **13**, 2187-2190.
60. Izzotti, A., Balansky R., Scatolini L., Rovida A. and De Flora S. (1995) Inhibition by *N*-acetylcysteine of carcinogen-DNA adducts in the tracheal epithelium of rats exposed to cigarette smoke. *Carcinogenesis*, **16**, 669-672.
61. Balansky, R., Izzotti A., Scatolini L., D'Agostini F. and De Flora S. (1996) Induction by carcinogens and chemoprevention by *N*-acetylcysteine of adducts to mitochondrial DNA in rat organs. *Cancer Res*, **56**, 1642-1647.
62. Arif, J.M., Cairola C.G., Glauert H.P., Kelloff G.J., Lubet R.A. and Gupta R.C. (1997) Effects of dietary supplementation of *N*-acetylcysteine on cigarette smoke-related DNA adducts in rat tissues. *Int J Oncol*, **11**, 1227-1233.

SAMENVATTING

In tabaksrook zijn meer dan 4000 chemische stoffen aanwezig waaronder 50 stoffen die mutageen en carcinogeen zijn gebleken in experimentele modellen en/of in de mens. Onder deze mutagene verbindingen bevinden zich de polycyclische aromatische koolwaterstoffen (PAK), aromatische amines en reactieve zuurstofverbindingen (ROS). Na blootstelling aan deze verbindingen zijn de volgende opeenvolgende stappen te onderscheiden; opname door het lichaam, biotransformatie in reactieve metabolieten, vorming van DNA interactie producten (DNA adducten), genetische veranderingen zoals oncogen-activatie en tumor-suppressorgen-inactivatie, en uiteindelijk de inductie van kwaadaardige tumoren. In het algemeen kan de tabaksrook gerelateerde carcinogene werking gevolgd worden door binnen deze keten van gebeurtenissen te monitoren, beginnend bij blootstelling aan tabaksrook tot de uiteindelijke ontwikkeling van tumoren. Deze benadering, ook wel biomonitoring genoemd, maakt gebruik van markers van blootstelling, markers van inwendige dosis, markers van moleculaire dosis, markers van preklinische effecten, markers van ziekte en markers van gevoeligheid. Markers die de gebeurtenissen aan het einde van deze keten weerspiegelen geven niet alleen een indruk van blootstelling maar tevens een indicatie van het risico. Idealiter zou biomonitoring plaats dienen te vinden in die organen waarin de tumor-vorming plaatsvindt (doelwitorganen). Echter, het is niet altijd mogelijk doelwitorganen zoals de long, te bestuderen vanwege de onmogelijkheid op routinematige basis weefsel hiervan te verkrijgen. Daarom wordt vaak gebruik gemaakt van surrogaatweefsels zoals witte bloedcellen. Een nadeel van het gebruik van surrogaatweefsel is, dat de gebeurtenissen hierin niet altijd representatief zijn voor hetgeen zich in het doelwitorgaan afspeelt. In onderhavige proefschrift is tabaksrook geïnduceerde carcinogeniteit bestudeerd in menselijk materiaal afkomstig van doelwit- en surrogaatweefsel door kwantificering van markers van moleculaire dosis zoals PAK-, aromatische amine- en ROS geïnduceerde DNA adducten.

Hoofdstuk 2 beschrijft een toepassing waarbij gebruik wordt gemaakt van geïnduceerd sputum, waarin zich cellen bevinden afkomstig van de lagere luchtwegen. Hoofdstuk 3 is een validatie studie waarbij DNA adduct metingen in geïnduceerd sputum vergeleken worden met die in perifere bloed lymfocyten. Hoofdstuk 4 beschrijft een studie waarin door immunohistochemische bepalingen in geïnduceerd sputum de relatieve bijdrage van PAK-DNA adducten en aromatische amine-DNA adducten met elkaar vergeleken wordt. Hoofdstuk 5 beschrijft een onderzoek waarin de relatie wordt bestudeerd van tabaksrook gerelateerde DNA adducten in geïnduceerd sputum en perifere bloed lymfocyten worden bestudeerd in relatie tot passieve blootstelling aan tabaksrook. In hoofdstuk 6 worden markers van oxidatieve DNA schade alsmede anti-oxidatieve verdedigingsmechanismen in rokers bestudeerd. In hoofdstuk 7 worden niveaus van DNA adducten vergeleken in verschillende locaties van de mondholte met verschillende gevoeligheidsprofielen voor kanker. Hoofdstuk 8 beschrijft een chemopreventieve studie met *N*-acetyl-L-cysteïne (NAC) in rokende vrijwilligers. In hoofdstuk 9 wordt tenslotte een overzicht gegeven van de uiteindelijke resultaten, samenvatting en aanbevelingen voor toekomstig onderzoek.

Geconcluserend kan worden dat biomonitoring van PAK- en aromatische amine-DNA adducten in doelwit- en relevant surrogaatweefsel, in het bijzonder geïnduceerd sputum, een waardevolle benadering is om tabaks-gerelateerde carcinogenese te bestuderen. Echter, de