# Mechanism of Oxidative Broncho-Epithelial Cell Damage with Reference to Gas Phase Cigarette Smoke

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**Abstract:** Tobacco smoke contains many thousands of chemicals including a large number of carcinogens. The exposure of human tissues and organs to these carcinogens and their metabolism in relation to smoking-related cancer has been reviewed. The assault on DNA and RNA as well as other cellular components by the ROS is the base for oxidation. Free radicals (ROS) are implicated in chemical carcinogenesis *via* various metabolic pathways. The participation of free radicals in tobacco smoke in the process of carcinogenesis is mainly due to the effect of oxidative substances on the signal transduction pathways which lead to the cell replication by transforming the signaling proteins. However, the exact mechanism through which free radicals function in this process is not completely understood.

**Keywords:** Cigarette smoke, oxidative stress, free radicals, COPD, lung cancer.

## INTRODUCTION

The exposure of human tissues and organs to tobacco smoke chemicals induces smoking-related diseases, such as chronic obstructive pulmonary disease (COPD) [1-4] or lung cancer [5,6]. The major cytotoxic effects of cigarette smoke on pulmonary and immune cells are attributed to the gaseous phase compounds [7-10]. There is a direct effect these compounds exercise on the most critical line of defense of the airway epithelium [9] and indirect evoking immune responses, which in turn have a deleterious effect on lung structure [11].

Most of these carcinogens undergo metabolic activation in mammalian tissues *via* oxidation [12]. Oxidative stress can be generated when the cell is subjected to an increased amount of endogenous and/or exogenous reactive oxygen species (ROS). Exogenous oxidative stress is a key mechanism by which cigarette smoke exerts its pathological effects. The ROS can attack almost any cellular structure or molecule interacting with and modifying, through oxidation, biologically important molecules, such as lipids, proteins and DNA.

The reaction of free radicals with cellular membranes leads to the formation of lipid hydroperoxides which are degraded into a variety of toxic products; chain reactions of lipid peroxidation are important causative agents of various diseases, including cancer. Protein oxidation by free radicals leads to oxidative alterations of aminoacid side chains and drastic cleavage of peptides by oxygen. The presence of carbonyl compounds in proteins shows that they have been subjected to oxidative damage by free radicals. DNA damage by ROS is mainly due to oxidized DNA bases causing miscoding. The ROS play an important role in the increase of 8-oxo-deoxy-guanosine, a miscoding adduct in the DNA of smokers' lungs. Oxidative DNA damage contributes to ROS-induced carcinogenesis. The ROS may also generate a cascade causing DNA nicking and single strand breaks.

Most of the volatile organic compounds of tobacco smoke gaseous phase exercise their toxic/carcinogenic effect *via* their metabolites [12]. The presence of a cytochrome $P_{450}$ system in Clara cells of the bronchial epithelium terminal bronchioles, is of broad toxicological significance. Many cytotoxic and/or carcinogenic chemicals require activation by this enzyme system. Thus, the Clara cells would be likely primary targets for the adverse effects of such chemicals and they may serve as major sites for production of toxic/carcinogenic metabolites that act on other cells [15].

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## HYDROCARBONS

### Isoprene

Isoprene (2-methyl-1,3-butadiene, or 2-methyl-buta-1,3-diene) is an important diolephin. The International Agency for Research on Cancer (IARC) has classified isoprene as a possible carcinogen in humans [13]. There is evidence that isoprene is also a carcinogen in rats [14]. Recently, Fabiani *et al*. supported evidence that isoprene is a possibly carcinogenic chemical in humans [15].

Isoprene is metabolized to its isomeric mono-epoxides by mono-oxygenase which is activated by the cytochrome P450. Mono-epoxides are further metabolized into the mutagenic di-epoxides of isoprene. There is supporting evidence that isoprene di-epoxides are highly strong bacterial mutagens [16]. The methyl group in the isoprene molecule is of extreme importance to biological activities. Studies on carcinogenesis related to isoprene have shown mutations in the ras-gene and in the form of A → G transactions [17,18]. According to Begeman *et al*. [19], N7-Gua-adducts as isoprene DNA adducts can be considered as a choice biomarker. In cigarette smoke nitrogen monoxide is oxidized to $NO_2$ which reacts with $O_2$ and form alkoxyl-radicals. According to Pryor [20,21], in the gas phase of cigarette smoke there is a mechanism of a steady-state condition which is based on NO chemistry. According to this mechanism, the first step is slow oxidation of NO from which the more active nitrogen dioxide is produced: $NO + 1/2\ O_2 \rightarrow NO_2$.

Nitrogen dioxide reacts rapidly with unsaturated substances which exist in smoke, such as isoprene and produces carbon-centered radicals ($R^{\bullet}$), which react with $O_2$ to produce peroxy-radicals:

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

Peroxy-radicals are converted into alkoxy-radicals after reacting with NO, and produce additional nitrogen dioxide ($NO_2$):

$$ROO^{\bullet} + NO \rightarrow RO^{\bullet} + NO_2$$

The reaction presented above shows that isoprene plays an important role in the production of toxic radicals in the gas phase of cigarettes smoke. This toxicity is mostly due to the continuous production of $NO_2$ and results in the constant production of toxic free radicals on a stable recyclable basis.

### Butadiene

Butadiene, an important chemical compound, is used for producing polymers and synthetic rubber. It is found in tobacco smoke and in car exhaust gases. The non-complete combustion of benzene in cars constitutes the major source of butadiene in the exhaust gases released into the air. It is a strong carcinogen when intro-

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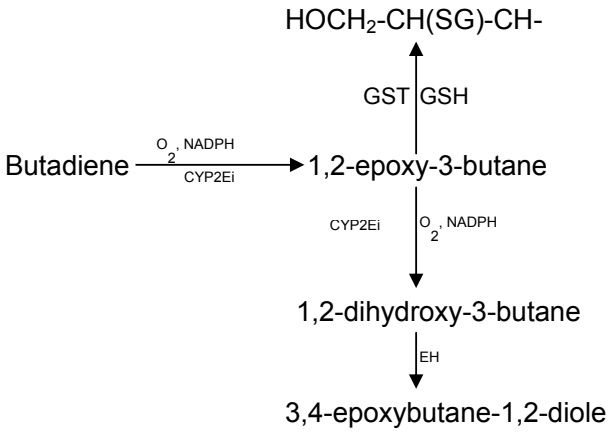


**Fig. (1).** Butadiene metabolism.

duced in mice [22,23] and a weak carcinogen in rats [24]. This means that difference in sensitivity can be interpreted by the existing metabolic differences in the two experimental animals. The chronic exposure of mice to butadiene inhalation creates lymphocytic lymphomas, hemangrosarcomas in the heart, alveolar/bronchial neoplasias, exfoliative neoplasms in the stomach, lobular-cell carcinomas in the breast.

Butadiene, following oxidation, in the presence of cytochrom $P_{450}$ is transversed to mono-epoxide, which is further oxidized to 1,2-dihydroxy-3-butene, and then further to 3,4-epoxybutane-1,2-diole. The epoxide metabolites of butadiene are responsible for its mitogenic and carcinogenic activities. Once butadiene is released into the environment, reactions occurring in the air transform this hydrocarbon into products that induce potentially greater adverse health effects than by the emitted hydrocarbon itself [25].

Butadiene metabolism has been studied in the microsomal systems of rats, mice and humans [26-29]. The isoenzyme of the cytochrom $P_{450}$ is probably responsible for the butadiene oxidation to butadiene monoxide. Butadiene metabolism (Fig. **1**) was first studied in the subcellular liver cell constituents of Wistar mice. Its mono-epoxide is inactivated *via* association with glutathione and in the presence of glutathione-S-transferase. During the inactivation of epoxy-butene, metabolites which are detected in the urine of people exposed to butadiene are created [30, 31], as well as in the urine of mice, rats, hamsters and monkeys. Diepoxy-butane is more toxic and 12 times more mitogenic at the hprt sites of the splenic T-cells of mice as compared to mono-epoxy-butene.

### Mutagenic Activities of Butadiene

The epoxide metabolites of butadiene are responsible for its mitogenic and carcinogenic activities [32,33]. Different epoxide metabolites are predominant cancer-initiating agents in cancer tests with butadiene, diepoxide in mice, and mono-epoxides in rats [34]. Two alkylation products of $N^7$-guanine are found in the liver DNA of mice and rats [34]. The *in vivo* mutagenic activity of butadiene is related to the K-ras gene activation by mutations in codon 13 (G→C transversion) found in lung and liver neoplasias as well as in lymphocytic lymphomas. This butadiene activity is expressed by tumor suppressor gene inactivation.

The $^{+}$G mutation frame shift is considered as a mutagenic "hot spot" for butadiene and its metabolites. Substitutions were observed in both GC and AT couples during exposure to the epoxides EB and DEB. The mutational spectrum for butadiene and the epoxides 1,2-epoxy-butene and diepoxy-butane is similar to that of ethylene oxide in $B_6C_3F_1$ mice. Consequently, 1,2-epoxy-butene and diepoxy-butane react with DNA deoxy-guanosine and produce $N^7$-(2-hydroxy-3-buten-1-yl)guanine and 7-(1-hydroxy-3-buten-2-yl)guanine [35, 36].

The loss of heterozygosity in chromosomes 4 and 11 has been found in more than 70% of mice exposed to butadiene, which developed breast and lung cancer. The alteration in chromosome 11 signifies the reduction of homozygosity at position $TrP_{53}$ in almost all lung cancers. The human chromosome 17 is a position where allele loss is often observed in a large spectrum of cancers in humans [37].

## AROMATIC HYDROCARBONS

### Benzene

Smoking has been incriminated as a unique and most important source of exposure to benzene for smokers [38,39] and is responsible for 50% of benzene exposure in the general population [38,39]. The gas phase of tobacco smoke contains 30.3-50.89 μg of benzene per cigarette. The complex metabolism of benzene proceeds *via* ring oxidation and ultimately ring cleavage. Benzene metabolism takes place quickly in the liver tissue where it is converted to benzene oxide which is hydrated to dihydrodiole, then oxidized to catechol and further to the semiquinone radical, which in turn reduces $O_2$ to the superoxide anion. The latter is dismutated to $H_2O_2$ leading to DNA damage. Benzene metabolism is dose-dependent and follows two pathways: the ring hydroxylation pathway and the open ring pathway [40, 41]. Hydroxylated metabolites form glycuronides or sulfuric conjugations which are excreted in the urine as detoxification products. The open-ring pathway leads to the formation of toxic by-products, trans-trans-myconaldehydes and myconic acid which are also detoxified and excreted in the urine [42].

Benzene oxide could also react with glutathione to form pro-mercapturic acid, or it is hydrated to form dihydrodiole and is oxidized to catechol (Fig. **2**). There is a rearrangement of its molecule forming phenol which forms hydroquinone.

Benzene is an established human hematotoxin with substantial interindividual variations in benzene-induced toxicity. Benzene metabolites are transferred from the liver to the bone marrow where they exercise their toxicity, associated with carrage molecules [43, 44]. Hydroquinone and o-benzoquinone are associated with the sulfhydryl groups of tubulin, in the mitotic spindle, thus impeding the appropriate formation of microtubules [45]. If there is no physiological function of the mitotic spindle, the dissociation of chromosomes becomes non feasible, thus aneuploidy is created [46].

Benzene and its metabolites may block iron incorporation into precursors of bone marrow cells [47]. Moreover, hydroquinone creates benzene toxicity by inhibiting cytokine production (ILa and ILb) from monocytes, which then negatively affect the regulation of hemopoiesis [48]. In mice exposed to benzene hydroquinone or benzoquinone, NO production is increased by the animals' white blood cells in the bone marrow as a response to inflammation messengers and growth factors [49].

NO has three different effects on the mitochondria of the B.M. cells: 1. inhibition of mitochondrial respiration, 2. stimulation in the production of oxidative substances and 3. causing mitochondria pore transition [50-52]. The inhibitions of respiration due to NO is caused by the reversible inhibition of cytochrome oxidase and by irreversible inhibition of different mitochondrial components from peroxynitrite and S-nitrosothiols [53, 54]. Investigation of the effect of benzene inhalation on the epithelial cells lining the respiratory tract, including bronchioles, terminal bronchioles, respiratory bronchioles and alveoli of male Sprague-Dawley rats has shown that inhalation of 300 ppm of benzene for 7 days may induce apoptotic changes in the parenchymal components in the lung [55].

The inhibition of respiration caused by NO results in cellular death and converting apoptosis to necrosis [47]. NO production represses cell proliferation and forms intermediate by-products which react with primary and secondary amines and forms nitrosa-

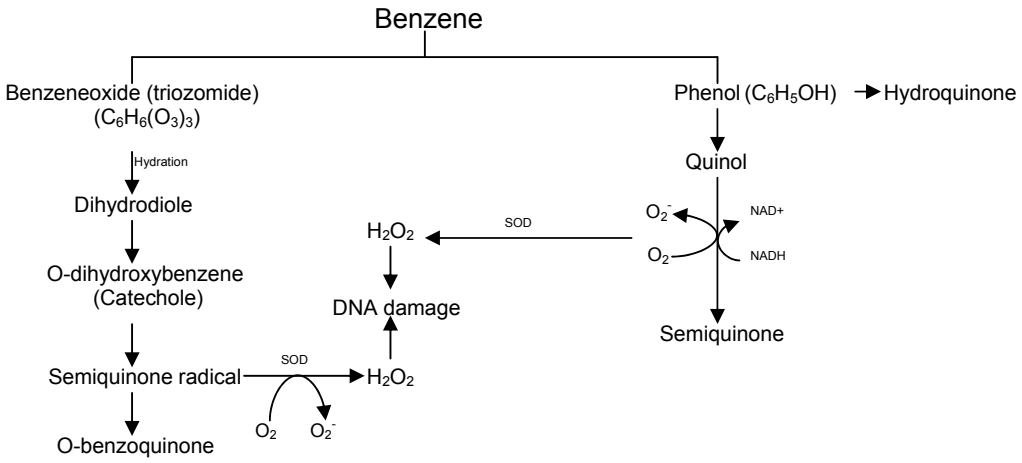


**Fig. (2).** Benzene metabolites and $H_2O_2$ in DNA damage.

**Acrolein affects transition factors KB and AP-1 (Fig…)**

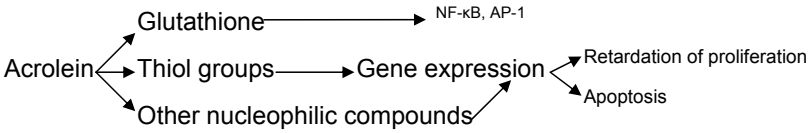


**Fig. (3).** Acrolein reacts directly with various genes and transcription factors, leading to retardation in cell proliferation rates and to possible apoptosis.

mines. Consequently, NO is important for the toxicity mechanism of benzene and its metabolites. Benzene metabolites are able to inhibit the function of enzymes that participate in DNA replication and reconstitution [56]. Both occupational exposure to the leukemogen benzene and *in vitro* exposure to its metabolite hydroquinone lead to the induction of numerical and structural chromosome changes. It creates chromosomal abnormalities suggestive of genetic damage, in both the blood cells and other tissue cells [57, 58].

A proportion of 90% of the tumors resulting from exposure to benzene show a loss of the functional "locus" of the $P_{53}$ allele. Loss of gene $P_{53}$ function is associated with genome instability and unregulated development [59, 60].

## Aldehydes

### *Acrolein*

Acrolein is formed *in vivo*, either following lipid peroxidation or from the oxidative substances present in tobacco smoke. Cigarette smoke contains 33-228 μg of acrolein per cigarette and this content depends on tobacco quality and smoking habits [61]. The acrolein levels in the air are 0.04-0.08 ppm, whilst its tobacco smoke concentrations are very high at about 90 ppm. During smoking, high local concentrations of acrolein are created in the respiratory pathway. It is a potent water-soluble electrophilic aldehyde, a highly dangerous substance to cells which inhibits the defense mechanism of the respiratory system [62]. *In vivo*, it is formed from the oxidative substances present in tobacco smoke and from the release of oxidative substances during inflammation [63, 64]. Acrolein affects some aminoacids (histidine, lysine, arginine) inducing covalent modifications to some proteins, advanced end-products of glycolysis or lipoxidation [65-68]. Certain end-products of glycolysis and advanced lipoxidation constitute agonists for the macrophage receptor. Macrophages are the main cell population for emphysema development [69]. Acrolein is present in tobacco smoke, in exhaust-gases and in the vapor of overheated cookers [70]. It binds quickly with nucleophylic substances such as glutathione. This activity of acrolein constitutes its very toxic background [71].

Acrolein induces the expression of glutathione-S-transferase [72]. Consequently, alterations due to acrolein may affect transcription pathways like those induced by oxidative stress [73]. Disturbance of the pro-oxidation-antioxidation equilibrium in favour of pro-oxidation results in remarkable damage [74]. Acrolein inhibits movements of the bronchoepithelial cell cilia, *in vitro*. Apoptosis of the bronchoepithelial cells is another mechanism of lung damage due to cigarette smoke. Acrolein induces apoptosis through the mitochondrial pathway that is mediated by ROS [75]. This cytotoxic activity is due to a reaction between the aldehydes and oxidative compounds in cigarette smoke [76]. The toxicity of aldehyde mixtures has revealed that histopathological alterations and cell proliferation of the olfactory epithelial cell-mixtures of formaldehyde, acetaldehyde and acrolein are the most serious and most extensive for both the respiratory and olfactory epithelia.

Acrolein may affect the inflammation mechanisms by aggregating neutrophiles and reducing their clearance by apoptosis *in vitro* [77]. Glutathione is a major target of acrolein when involved in the control of apoptosis and it is a factor that relates acrolein with death. Acrolein has an immediate and prompt effect on cellular GSH, and is based on the redox regulation of NF-kB, it is expected to influence its activation directly or indirectly, through changes of its content in GSH and more directly through its joining with the nucleophilic cysteine present in the subunits $P_{50}$ or $P_{65}$ [78, 79]. Cysteine sulfydryl groups are the primary soft nucleophilic targets of acrolein [80, 81]. Antioxidative compounds activate AP-1 [82, 83]. While AP-1 and NF-kB respond inversely to antioxidative compounds, the oxidants play a positive role in the activation process of both factors [84] (Fig. **3**).

$P_{53}$ activity is controlled at many levels by the cellular thiol-redox system. Acrolein alters this control by its binding to cysteines, thus preventing $P_{53}$ from binding to the specific target molecules or by altering the total of reduced thioles. The joining of $P_{53}$ with DNA and all transcription activities depends on the presence of cysteines in the joining with DNA sites [85]. Moreover, increased reduction of the cell thioles by *N*-acetyl-cysteine increases the expression of $P_{53}$ by increasing the transcription rate of the $P_{53}$

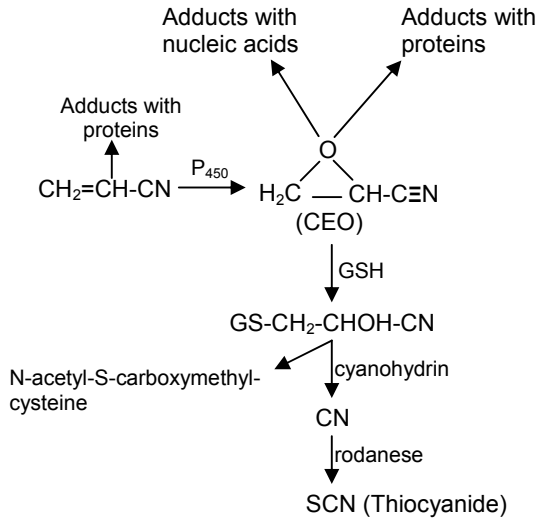


**Fig. (4).** The metabolic pathways of acrylonitrile. CEO = Cyanoethyl oxide, GSH = Glutathione.

mRNA [86]. Thus, $P_{53}$ activity is controlled at many levels by the cellular thiol-redox system. Acrolein exposure results in metalloproteinase secretion from macrophages *via* a mechanism that involves an increase in [$Ca^{2+}$] (I), leading to xanthine oxidase activation and an increase in ROS production [87, 88].

The thioredoxin system is very important for the maintenance of cellular thiol redox balance, and is critical for cell survival. Normally, thioredoxin reductase maintains the cytosolic thioredoxon-1 and mitochondrial thioredoxin-2 in the reduced state, and the thioredoxins keep the peroxiredoxins reduced. The effects of acrolein on thioredoxin reductase, thioredoxin and peroxiredoxins in human bronchial epithelial cells have been determined. The effects of acrolein on the thioredoxin system and peroxyredoxins could have important implications for cell survival, redox-sensitive cell signaling, and tolerance to other oxidant insults [84]. Transcription levels of several members of the metallothionein class of cytoprotective metal-chelating proteins decreased strongly in response to acrolein [85]. The effect of acrolein on the c-myc gene is absolutely necessary for proliferation and apoptosis. The c-myc gene plays a role in both the inhibition and activation of transcription.

## Acetaldehyde

Acetaldehyde is carcinogenic in experimental animals. It has been classified in the 2B group [79]. Direct exposure to acetaldehyde present in the gas phase of cigarette smoke can induce paralysis of the ciliary beat of the bronchoepithelial cells. Acetaldehyde toxicity is due to the polarity of the carbonyl groups, something which lends nucleophilic properties to it, when it is added to substances with amines, such as proteins and DNA, with which adducts are formed. In the gas phase of cigarette smoke, acetaldehyde behaves as a base, due to its proton relationship. Paralysis of the cilia beats occurs during 4 hours exposure of the bronchoepithelial cells to acetaldehyde present in the gaseous phase of cigarette smoke [89]. This cilia malfunction is related to the direct inactivation of the cilia ATPase and also to adduct formation with the dienine and tubulin amino group of the cilia [90-92]. When animals or humans are exposed to acetaldehyde or cigarette smoke there is clearance inhibition of the airways [93].

The result of this acetaldehyde action on the bronchoepithelial cell cilia is considerably increased by the action of cyanamide which is contained in cigarette smoke and which excludes the action of acetaldehyde-dehydrogenase and thus brings about a more intense cilia stasis [94]. In addition, the acetaldehyde of cigarette smoke can activate protein kinase-C resulting in IL-8 release under inflammatory conditions during which the activated component of the complement C5 (C5a) is increased in the air ducts, and which is responsible for the increased release of IL-8. Acetaldehyde is able to inhibit DNA reconstitution and the strengthening of mutagenesis in cultured human cells [95], and it also contributes to bronchogenic cancer development from cigarette smoke in humans [96]. The main metabolic pathway is the oxidation of its aldehyde component to acetic acid in the presence of aldehyde-dehydrogenase (ALDH). The latter, is localized in the respiratory epithelium of rats. Acetaldehyde is genotoxic *in vitro* and it induces gene mutations in mammalian cells. There is evidence that acetaldehyde is able to induce cross-linking of protein with DNA (protein-DNA or DNA-DNA).

## Nitriles

### *Acrylonitrile*

Humans are exposed to acrylonitrile when it is used in the synthesis of different organic substances, such as acrylic fibers and plastics. Tobacco smoke includes acrylonitrile in such a quantity that it is able to damage human health.

Acrylonitrile acts through its metabolites. Its most interesting pathway of metabolism is the direct joining with glutathione and epoxidation to form cynoethylene oxide (CEO), two reactions catalyzed by cytochrome $P_{450}$. Acrylonitrile enhances lipid peroxidation as indicated by malondialdehyde (MDA) accumulation. Reduced glutathione (GSH) level was ascertained in cytotoxicity experiments in rats [97]. However, antioxidants do not prevent acrylonitrile toxicity

Recent studies have shown that acrylonitrile is a weak carcinogen [98-100]. The International Agency for Research on Cancer (IARC) has classified acrylonitrile as a "possible carcinogen for humans" (group 2B) [101]. Oxidative stress is a possible mechanism of tumor development due to acrylonitrile [102, 103]. Cyanide induces lipid peroxidation in the rat brain [104]. In a summary of currently available published studies on lung cancer and occupational acrylonitrile exposure, a possible contribution of smoking confounding the increased risk was not excluded [105]. Oxygen radicals which are formed in the gaseous phase of cigarette smoke (cs) may contribute to oxidative DNA damage and tumor development. The quantities of acrylonitrile in cs vary from 10-20 μg per cigarette [106].

Acrylonitrile metabolism is furthered *via* two different pathways: The first pathway leads to the direct joining with glutathione and epoxidation to form cyanoethylene epoxide (CEO) (Fig. **4**). N-acetyl-S-(2-cyanoethyl) cysteine is the main metabolite which is formed by the direct reaction of acrylonitrile with glutathione [107-109]. The second important pathway of acrylonitrile metabolism that leads, *via* epoxidation, to 2-cyanoethylene oxide, is furthered by Cyt $P_{450}$ [110]. Acrylonitrile and ethylene oxide can be joined with proteins [111] and react with tissue thioles, resulting in a quick depletion of the induced glutathione reserves in different tissues. Lung epithelial cells are capable of metabolizing acrylonitrile and ethylene oxide [112].

### *Toxicity in Humans*

The toxicity of acrylonitrile is due to its two metabolic characteristics: cyanide formation and depletion of cellular glutathione reservoirs [113]. Acrylonitrile is able to create gene mutations, chromosomal deletions, non-programmed DNA synthesis and cell transformation. All these side effects demand previous metabolic transformation of acrylonitrile.

### *Furan*

Furan is a cyclic dien-ether in liquid form; it enters the human organism through the respiratory pathway. It is present in tobacco smoke and exhaust gases. Furan is metabolically activated by cyto-

chrome $P_{450}$ into a cytotoxic product which stimulates cell replication [114,115]. It is considered to be a carcinogen in humans. However, there are sufficient data to substantiate its carcinogenic activity in humans. In bacteria, furan can induce gene mutations [116, 117]. *In vivo* studies, in the ovary cells of Chinese hamsters, furan induces DNA damage [101]. In *in vivo* experiments in mammals, furan induces chromosomal deletion in the bone marrow cells of $B_6C_3F_1$ mice [118].

### *Overview*

The assault on DNA and RNA as well as other cellular components by the ROS which are included or produced by cigarette smoke, is the base for cancer development from oxidation. Free radicals (ROS) are implicated in chemical carcinogenesis *via* various metabolic pathways [119, 120]. The participation of free radicals in tobacco smoke in the process of carcinogenesis is mainly due to the effect of oxidative substances on the signal transduction pathways which lead to the cell replication by transforming the signaling proteins.

Oxidative stress actually induces phosphorylation and activation of the many proteins involved in signal transduction to the cell nucleus. Members of the MAP kinase family [121-124] kinase MEK and Ras [125] are included in these proteins as well as some growth factor receptors, such as platelet growth factor receptor (PDGF) [126] and the epidermal growth factor receptor (EGFR) [127-129].

Even if the mechanism through which free radicals perform these conversions to the proteins which transfer information are not completely understood, there is, however, supporting evidence that increased phosphorylation of the epidermal growth factor receptor (EGFR) by $H_2O_2$ is the result of deactivation of the tyrosine phosphatases [128, 130]. This denotes that $H_2O_2$ exerts an inhibitive activity on the cellular mechanisms which limit the information transfer through the growth factor. The above down-regulation of the activated receptors is important, because cell transformation and tumor development takes place because cells are unable to promote the endocytosis caused by the receptor binders [131, 132]. The oxidative stress through $H_2O_2$ in cigarette smoke quickly inhibits EGF receptor endocytosis [133, 134].

Despite the fact that free radicals are the main cause of certain cancers, it is highly unlikely that they are necessary in all cases, since alternative initial pathways exist, as well as mechanisms for the promotion of carcinogenesis [135]. There are, however, serious indications with regard to the role of free radicals in the promotion of the carcinogenic processes [136, 137].

### *Epilogue*

Tobacco smoke contains many thousands of chemicals including a large number of carcinogens. Most of the carcinogens undergo metabolic activation in mammalian tissues *via* oxidation. The exposure of human tissues and organs to these carcinogens and their metabolism is an important mechanism by which smoking-related cancer is initiated.

## REFERENCES


[1] Hogg, J.C.; Chu, F.; Utokaparch, S.; Woods, R.; Elliott, W.M.; Buzatu, L.; Cherniack, R.M., Rogers, R.M.; Sciurba, F.C.; Coxson, H.O.; Paré, P.D. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N. Engl. J. Med.*, **2004**, *350*, 2645-2653.

[2] Kim, V.; Rogers, T.J.; Criner, G.J. New concepts in the pathobiology of chronic obstructive pulmonary disease. *Proc. Am. Thorac. Soc.*, **2008**, *5*, 478-485.

[3] Chrirg, A.; Tai, H.; Coultard, T.; Wang, R.; Wright, J.L. Cigarette smoke drives small airway remodeling by induction of growth factors in the airway wall. *Am. J. Resp. Crit. Care Med.*, **2006**, *174*, 1327-1334.

[4] Ning, W.; Li, C.J.; Kaminski, N.; Feghali-Bostwick, C.A.; Alber, S.M.; Di Y.P.; Otterbein, S.L.; Song, R.; Hayashi, S.; Zhou, Z.; Pinsky, D.J.; Watkins, S.C.; Pilewski, J.M.; Sciurba, F.C.; Peters, D.G.; Hogg, J.C.; Choi, A.M. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 14895-14900.

[5] Stavridis, J.C. Lung carcinogenesis: pivotal role of metals in tobacco smoke. *Free Radic. Biol. Med.*, **2006**, *41*, 1017-1030.

[6] Hecht, S.S. Tobacco smoke carcinogens and lung cancer. *J. Natl. Cancer Inst.*, **1999**, *91*, 1194-1210.

[7] Yamaguchi, Y.; Nasu, F.; Harada, A.; Kunimoto, M. Oxidants in the gas phase of cigarette smoke pass through the lung alveolar wall and raise systemic oxidative stress. *J. Pharmacol. Sci.* **2007**,*103*, 275-282.

[8] Pouli, A.E.; Hatzinikolaou, D.G.; Piperi, C.; Stavridou, A.; Psalidopoulos, M.C.; Stavridis, J.C. The cytotoxic effect of volatile organic compounds of the gas phase of cigarette smoke on lung epithelial cells. *Free Radic. Biol. Med*. **2003**, *34*, 345-355.

[9] Sdralia, N.D.; Patmanidou, A.L.; Velentzas, A.D.; Margaritis, L.H.; Hatzinikolaou, D.G.; Baltatzis, G.E.; Stavridou, A.J. The mode of lymphoblastoid cell death in response to gas phase cigarette smoke is dose-dependent. *Respir. Res. J*. **2009**, *10*, 82.

[10] Betsuyaku, T.; Hamamura, L.; Hata, J.; Takahashi, H.; Mitsuhasi, H.; Adair-Kirk, T.L.; Senior, R.H.; Nishimura, M. Bronchiolar chemokine expression is different after single versus repeated cigarette smoke exposure. *Respir. Res.* **2008**, *9*, 7-18.

[11] Giordano, R.J.; Lahdentrate, J.; Zhen, L.; Chukwueke, U.; Petrache, I.; Langley, R.R.; Fidler, I.J.; Pasqualini, R.; Tuder, R.M.; Arap, W. Targeted induction of lung endothelial cell apoptosis causes emphysema-like changes in the mouse. *J. Biol. Chem.* **2008**, *283*, 29447-29460.

[12] Stavridis, J.C. *Oxidation: The Cornerstone of Carcinogenesis*. Springer Science, **2008**.

[13] IARC, Isoprene. In: *IARC Monographs on the Evaluation of the Carcinogenic Risks of Chemicals to Humans: Some Industrial Chemicals.* IARC: Lyon, France, **1994**, vol. *60*, pp. 215-232.

[14] Osterman-Golkar, S.; Peltoken, K.; Auttinen-Klemetti, T.; Landin, H.H.; Zorcec, V.; Sorsa, M. Haemoglobin adducts as biomarkers of occupational exposure to 1,3-butadiene. *Mutagenesis*, **1996**, *11*, 145-149.

[15] Fabiani, R.; Rosignoli, P.; DeBartolomeo, A.; Fucceli, R.; Morozzi, G. DNA-damaging ability of isoprene and isoprene mono-epoxide (EPOX I) in human cells evaluated with the comet assay. *Mutat. Res.* **2007**, *629*, 7-13.

[16] Cervasi, P.G.; Citti, L.; Del Monte, M.; Longo, V.; Benetti, D. Mutagenicity and chemical reactivity of epoxidic intermediates of the isoprene metabolism and other structurally related compounds. *Mutat. Res.* **1985**, *156*, 77.

[17] Goodrow, T.L.; Nichols, W.W. Storer, R.D.; Anderson, M.W.; Maronpot, R.R. Activation of H-ras is prevalent in 1,3-butadiene-induced and spontaneously occurring murine Harderian gland tumors. *Carcinogenesis* **1994**, *15*, 2665-2667.

[18] Hong, H.L.;Devereux, T.R.; Melnick, R.L.; Eldridge, S.R.; Greenwell, A.; Haseman, J.; Boorman, G.A.; Sills, R.C. Both K-ras and H-ras protooncogene mutations are associated with Harderian gland tumorigenesis in B6C3F1 mice exposed to isoprene for 26 weeks. *Carcinogenesis* **1997**, *18*, 783-789.

[19] Begeman, P.; Christova-Georgieva, N.L.; Sangaiah, R.; Koc, H.; Zhang, D.; Golding, B.T.; Gold, A.; Swenberg, J.A. Synthesis, characterization, and identification of N7-guanine adducts of isoprene monoepoxides *in vitro*. *Chem. Res. Toxicol*. **2004**, *17*, 929-936.

[20] Pryor, W.A. Cigarette smoke and the involvement of free radical reactions in chemical carcinogenesis. *Br. J. Cancer* **1987**, *55*,19-23.

[21] Church, D.F.; Pryor, W.A. Cigarette smoke and the involvement of free radical reactions in chemical carcinogenesis. *Environ. Health Perspect*. **1985**, *64*, 111-126.

[22] Melnick, R.L.; Huff, J.E.; Roycroff, J.H.; Chon, B.J.; Miller, R.H. Inhalation toxicology and carcinogenicity of 1,3-butadiene in B6C3F1 mice following 65 weeks of exposure. *Environ. Health Perspect*. **1990**, *86*, 27-36.

[23] National Toxicology Program, In Draft of the National Toxicology Program TR No 434, *National Institute of Health*, Bethesda, MD, **1993**.

[24] Owen, P.E.; Glaister, J.R.; Gaunt, I.F.; Pullinger, D.H. Inhalation toxicity studies with 1,3-butadiene. 3. Two year toxicity/carcinogenicity study in rats. *Am. Industr. Hugg. Assod. J*. **1987**, *48*, 407-413.

[25] Doyle, M.; Sexton, K.G.; Jeffries, H.; Bridge, K.; Jaspers, I. Effects of 1,3-butadiene, isoprene, and their photochemical degradation products on human lung cells. *Environ. Health Perspect*. **2004**, *112*, 1488-1495.

[26] Schmidt, U.; Loeser, E. Neoplastic and nonneoplastic lesions in aging female rats with special reference to the functional morphology of the hyperplastic and neoplastic changes in the pituitary gl. *Arch. Toxicol*. **1985**, *57*, 77-83.

[27] Elfarra, A.A.; Duescher, R.J.; Pasch, C.M. Mechanisms of 1,3-butadiene oxidations to butadiene monoxide and crotonaldehyde by mouse liver microsomes and chloroperoxidase. *Arch. Biochem. Biophys*. **1991**, *286*, 244-251.

[28] Csanady, G.A.; Guengerich, F.F.; Bond, J.A. Comparison of the biotransformation of 1,3-butadiene and its metabolite, butadiene monoepoxide, by hepatic and pulmonary tissues from humans, rats and mice. *Carcinogenesis* **1992**, *13*, 1143-1153.

[29] Sharer, J.E.; Duescher, R.J.; Elfarra, A.A. Species and tissue differences in the microsomal oxidation of 1,3-butadiene and the glutathione conjugation of butadiene monoxide in mice and rats. Possible role in 1,3-butadiene-induced toxicity. *Drug Metab. Dispos*. **1992**, *20*, 658-664.

[30] Sabourin, P.J.; Burka, L.T.; Bechtold, W.E.; Dahl, A.R., Hoover, M.D.; Chang, I.Y.; Henderson, R.F. Species differences in urinary butadiene metabolites; identification of 1,2-dihydroxy-4-(N-acetylcysteinyl)butane, a novel metabolite of butadiene. *Carcinogenesis* **1992**, *13*, 1633-1638.

[31] Bechtold, W.E.; Stunk, M.R.; Chang, I.Y.; Ward, J.B. Jr; Henderson, R.F. Species differences in urinary butadiene metabolites: comparisons of metabolite ratios between mice, rats, and humans. *Toxicol. Appl. Pharmacol.* **1994**, *127*, 44-49.

[32] Placke, M.E.; Griffis, L.; Bird, M.; Bus, J.; Persing, R.L.; Cox, L.A .Jr. Chronic inhalation oncogenicity study of isoprene in B6C3F1 mice. *Toxicology*, **1996**, *113*, 253.

[33] National Toxicology Program (NTP), In: NTP Technical Report on the Toxicology and Carcinogenesis Studies. *NIH Publication*s. No 99-39-76, **1999**.

[34] Fred, C.; Tornqvist, M.; Granath, F. Evaluation of cancer tests of 1,3-butadiene using internal dose, genotoxic potency, and a multiplicative risk model. *Cancer Res.* **2008**, *68*, 8014-8021.

[35] Jelitto, B.; Vangala, R.R.; Laib, R.J. Species differences in DNA damage by butadiene: role of diepoxybutane. *Arch. Toxicol. Suppl*. **1989**, *13*, 246-249.

[36] Van Duren, B.I. Carcinogenic epoxide lactones and halo-ethers and their mode of action. *Ann. N. Y. Acad. Sci*. **1969**, *163*, 633-651.

[37] Serzinger, B.R.; Klinger, H.P.; Junien, C.; Nakamura, Y.; Lebeau, M.; Cavenee, W.; Emmanuel, B.; Ponder, B; Naylor, S.; Mitelman, F.; Louis, D.; Men_9 A.; Newsham, I.; Decker, J.; Kaebling, M.; Henry, I.; von Deimling, A. Eleventh Workshop on Human Gene Mapping. Report of the committee on chromosome and gene loss in human neoplasia. *Cytogenet. Cell, Cell Genet*. **1991**, *58*, 1080-1096.

[38] Wallace, L.A. Major sources of benzene exposure. *Environ. Health Perspect.* **1989a**, *82*, 165-169.

[39] Wallace, L.A. The exposure of the general population to benzene. *Cell Biol. Toxicol*. **1989b**, *5*, 297-314.

[40] Sabourin, P.J.; Bechtold, W.E.; Birnbaum, L.S.; Lucier, G.; Henderson, R.F. Differences in the metabolism and disposition of inhaled [3H]benzene by F344/N rats and B6C3F1 mice. *Toxicol. Appl. Pharmacol*. **1988**, *94*, 128-140.

[41] Snyder, R.; Witz, G.; Goldstein, B.D. The toxicology of benzene. *Environ. Health Perspect*. **1993b**, *100*, 293-306.

[42] Latrianot, L.; Goldstein, b.D.; Witz, G. Formation of muconaldehyde, an open-ring metabolite of benzene, in mouse liver microsomes: an additional pathway for toxic metabolites.*Proc. Natl. Acad. Sci. USA.* **1986**, *83*, 8356-8360.

[43] McDonald, T.A.; Yeowell O' Connel, K.; Rappaporti, S.M. Comparison of protein adducts of benzene oxide and benzoquinone in the blood and bone marrow of rats and mice exposed to [14C/13C6]benzene. *Cancer Res.* **1994**, *54*, 4907-4914.

[44] Bechtold, W.E.; Henderson, R.F. Biomarkers of human exposure to benzene. *J. Toxicol. Environ. Health* **1993**, *40*, 377-386.

[45] Irons, R.D. Quinones as toxic metabolites of benzene. *J. Toxicol. Environ. Health* **1985**, *16*, 673-678.

[46] Robertson, M.L.; Eastmond, D.A.; Smith, M.T. Two benzene metabolites, catechol and hydroquinone, produce a synergistic induction of micronuclei and toxicity in cultured human lymphocytes. *Mutat. Res.* **1991**a, *249*, 201-210.

[47] Snyder, R.; Dimitriadis, E.; Guy, R.; Hu, P.; Cooper, K.; Bauer, H.; Witz, G.; Goldstein, B.D. Studies on the mechanism of benzene toxicity. *Environ. Health Perspect*. **1989**, *82*, 31-35.

[48] Miller, A.C.; Schattenberg, D.D.; Malkinson, A.M.; Ross, D. Decreased content of the IL1 alpha processing enzyme calpain in murine bone marrow-derived macrophages after treatment with the benzene metabolite hydroquinone. *Toxicol. Lett.* **1994**, *74*, 177-184.

[49] Laskin, J.D.; Rao, N.R.; Prinhabi, C.J.; Laskin, D.L.; Synder, R. Distinct actions of benzene and its metabolites on nitric oxide production by bone marrow leukocytes. *J. Leukoc. Biol*. **1995**, *57*, 422-426.

[50] Borntaite, V.; Morkuniene, R.; Brown, G.C. Nitric oxide donors, nitrosothiols and mitochondrial respiration inhibitors induce caspase activation by different mechanisms. *FEBS Lett*. **2000**, *467*, 155-159.

[51] Poderoso, J.J.; Carreras, M.C.; Lisdero, C.; Casrreras, M.C; Radisic, M.; Schöpfer, F.; Cadenas, E.; Boveris, A. Nitric oxide regulates oxygen uptake and hydrogen peroxide release by the isolated beating rat heart. *Am. J. Physiol*. **1998**, *274*, C112-C119.

[52] Poderoso, J.J.; Carreras, M.C.; Hisdero, C.; Riobo, N.; Schopfer, F.; Boveri, A. Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles. *Arch. Biophys. Biochem*. **1996**, *328*, 85-92.

[53] Brown, G.C.; Cooper, C.E. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett.* **1994**, *356*, 295-298.

[54] Clementi, E.; Brown, G.L.; Feelisch, M.; Moncada, S. Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc. Natl. Acad. Sci. USA.* **1998**, *95*, 7631-7636.

[55] Weaver, C.V.; Liu, S.P.; Lu, J.F.; Lin, B.S. The effects of benzene exposure on apoptosis in epithelial lung cells: localization by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) and the immunocytochemical localization of apoptosis-related gene products. *Cell Biol. Toxicol*. **2007**, *23*, 201-220.

[56] Chen, H.; Eastmond, D.A. Topoisomerase inhibition by phenolic metabolites: a potential mechanism for benzene's clastogenic effects. *Carcinogenesis* **1995**, *16*, 2301-2367.

[57] Tice, R.R.; Costa, D.L.; Drew, R.T. Cytogenetic effects of inhaled benzene in murine bone marrow: induction of sister chromatid exchanges, chromosomal aberrations, and cellular proliferation inhibition in DBA/2 mice. *Proc. Natl. Acad. Sci. USA.* **1980**, *77*, 2148-2152.

[58] Tice, R.R.; Vogt, T.F.; Costa, D.L. Cytogenetic effects of inhaled benzene in murine bone marrow. *Environ. Sci. Res.* **1982**, *25*, 257-275.

[59] Livingstone, L.R.; White, A.; Spouse, J.; Livanos, E.; Jacks, T.; Tisty, T.D. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell* **1992**, *70*, 923-935.

[60] Harvey, M.; Sands, A.T.; Weis, R.S.; Hegi, M.E.; Wiseman, R.W.; Pantazis, P.; Giovanella, B.C.; Tainsky, M.A.; Bradley, A.; Donehower, L.A. *In vitro* growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene* **1993**, *8*, 2457-2467.

[61] Environmental Health Criteria, 127, *Acrolein*, Geneva: WHO, **1992**.

[62] Li, L.; Holian, A. Acrolein: a respiratory toxin that suppresses pulmonary host defense. *Rev. Environ. Health* **1998**,*13*, 99-108.

[63] Esterbauer, H.; Schauer, R. J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med*. **1991**, *11*, 81-128.

[64] Frei, B.; Forte, T.M.; Ames, B.N.; Cross, C.E. Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. *Biochem. J*. **1991**, *277*(Part I), 133-138.

[65] Cerami, C.; Founds, H.; Nicholl, I.; Mitsuhashi, T.; Giordano, D.; Vanpatten, S.; Lee, A.; Al-Abed, Y.; Vlassara, H.; Bucala, R.; Cerami, A. Tobacco smoke is a source of toxic reactive glycation products. *Proc. Natl. Acad. Sci. USA.* **1997**, *94*, 13915-13920.

[66] Dean, R.T.; Fu, S.; Stocker, R.; Davies, M.J. Biochemistry and pathology of radical-mediated protein oxidation. *Biochem. J*. **1997**, *324*(Part I): 1-18.

[67] Refsgaard, H.H.; Tsai, L.; Stadtman, E.R. Modifications of proteins by polyunsaturated fatty acid peroxidation products. *Proc. Natl. Acad. Sci. USA*. **2000**, *97*, 611-616.

[68] Uchida, K.; Kanematsu, M.; Sakai, K.; Matsuda, T.; Hattori, N.; Mizuno, Y.; Suzuki, D.; Miyata, T.; Noguchi, N.; Niki, E.; Osawa, T. Protein-bound acrolein: potential markers for oxidative stress. *Proc. Natl. Acad. Sci. USA*. **1998**, *95*, 4882-4888.

[69] Shapiro, S.D. The macrophage in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med*. **1999**, *160*, S29-32.

[70] Bauchamp, R.D.; Jr., Andjelkovich, D.A.; Kligerman, A.D.; Morgan, K.T.; Heck, H.D. A critical review of the literature on acrolein toxicity. *Crit. Rev. Toxicol*. **1985**, *14*, 309-380.

[71] Sierra, L.M.; Barros, A.R.; Garcia, M.; Ferreiro, J.A.; Comendator, M.A. Acrolein genotoxicity in Drosophila melanogaster. I. Somatic and germinal mutagenesis under proficient repair conditions. *Mutat. Res.* **1991**, *260*, 247-256.

[72] Uchida, K. Current status of acrolein as a lipid peroxidation product. *Trends Cardiovasc. Med*. **1999**, *9*, 109-113.

[73] Costa, D.L.; Amdur, M.O. In *Casarett and Doul's Toxicology*, 5th ed., **1996**, pp. 857-882.

[74] Sies, H. *Oxidative Stress: Introsuction in Oxidative Stress, Oxidants and Antioxidants*; Sies, H Ed.; Academic Press: New York, **1991**, pp. 15-20.

[75] Roy, J.; Pallepati, P.; Bettaieb, A.; Tanel, A.; Averill-Bates, D.A. Acrolein induces a cellular stress response and triggers mitochondrial apoptosis in A549 cells. *Chem. Biol. Interact*. **2009**, *181*, 154-167.

[76] Hoshimo, Y.; Mio, T.; Nagai, S.; Miki, H.; Ito, I.; Izumi, T. Cytotoxic effects of cigarette smoke extract on an alveolar type II cell-derived cell line. *Am. J. Physiol. Lung Cell Mol. Physiol*. **2001**, *281*, L509-L516.

[77] Finkelstein, E.I.; Nardini, M.; Van der Vlret, A. Inhibition of neutrophil apoptosis by acrolein: a mechanism of tobacco-related lung disease? *Am. J. Physiol. Lung Cell Mol. Physiol*. **2001**, *28*, L732-L739.

[78] Kukiar, S.; Rabson, A.B.; Gelinas, C. The RxxRxRxxC motif conserved in all Rel/kappa B proteins is essential for the DNA-binding activity and redox regulation of the v-Rel oncoprotein. *Mol. Cell. Biol*. **1992**, *12*, 3094-3106.

[79] Toledano, M.B.; Ghosh, D.; Trink, F.; Leonard, W.J. N-terminal DNA-binding domains contribute to differential DNA-binding specificities of NF-kappa B p50 and p65. *Mol. Cell. Biol*. **1993**,*13*, 852-860.

[80] Lopachin, R.M.; Gavin, T.; Petersen, D.R.; Barbers, D.S. Molecular mechanisms of 4-hydroxy-2-nonenal and acrolein toxicity: nucleophilic targets and adduct formation. *Chem. Res. Toxicol*. **2009**, *22*, 1499-1508.

[81] Cai, J.; Bhatnagar, A.; Pierce, W.M.Jr. Protein modification by acrolein: formation and stability of cysteine adducts. *Chem. Res. Toxicol*. **2009**, *22*, 708-716.

[82] Meyer, M.; Schreck, R.; Baenerle, P.A. $H_2O_2$ and antioxidants have opposite effects on activation of NF-kappa B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J*. **1993**,*12*, 2005-2015.

[83] Schenk, H.; Klein, M.; Erdbrugger, N.; Droge, W.; Schulze-Osthoff, K. Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-kappa B and AP-1. *Proc. Natl. Acad. Sci. USA.* **1994**, *91*, 1672-1676.

[84] Pinkus, R.; Weinwe, L.M.; Daniel, V. Role of oxidants and antioxidants in the induction of AP-1, NF-kappaB, and glutathione S-transferase gene expression. *J. Biol. Chem.* **1996**, *271*, 13422-13429.

[85] Hainant, P.; Milner, J. Redox modulation of p53 conformation and sequence-specific DNA binding *in vitro*. *Cancer Res.* **1993**, *53*, 4469-4473.

[86] Liu, M.; Pellin, J.C.; Ju, J.; Chu, E.; Brash, D.E. Antioxidant action *via* p53 mediated apoptosis. *Cancer Res.* **1998**, *58*, 1723-1729.

[87] Myers, C.R.; Myers, J.M. The effects of acrolein on peroxiredoxins, thioredoxins, and thioredoxin reductase in human bronchial epithelial cells. *Toxicology* **2009**, *257*(1-2), 95-104.

[88] Thomson, C.A.; Bureham, P.C. Genome-wide transcriptional responses to acrolein. *Chem. Res. Toxicol.* **2008**, *21*, 2245-2256.

[89] Sisson, J.H.; Tuma, D.J. Vapor phase exposure to acetaldehyde generated from ethanol inhibits bovine bronchial epithelial cell ciliary motility. *Alcohol Clin. Exp. Res.* **1994**, *18*, 1252-1255.

[90] Donohue, T.M.; Tuma, D.J.; Jennet, R.B.; Sorell, M.F. Binding of metabolically derived acetaldehyde to hepatic proteins *in vitro*. *Lab. Invest.* **1983**, *49*, 226-229.

[91] Tuma, D.J.; Jennet, R.B.; Sorrell, M.F. The interaction of acetaldehyde with tubulin. *Ann. N. Y. Acad. Sci.* **1987**, *492*, 277-286.

[92] Lin, R.C.; Smith, R.S.; Lumeng, L.J. Detection of a protein-acetaldehyde adduct in the liver of rats fed alcohol chronically. *J. Clin. Invest.* **1988**, *81*, 615-619.

[93] Sisson, J.H.; Tuma, D.J.; Rennard, S.I. Acetaldehyde-mediated cilia dysfunction in bovine bronchial epithelial [published errata appear in Am J Physiol 1992 Feb;262(2 Pt 1):section L following table of contents and 1992 Jun;262(6 Pt 3):section L following table of contents]. *Am. J. Physiol.* **1991**, *260*(2 Pt 1), L29-L36.

[94] Shirota, F.N.; Demaster, E.G.; Kwon, C.H.; Nagasawa, H.T. Metabolism of cyanamide to cyanide and an inhibitor of aldehyde dehydrogenase (ALDH) by rat liver microsomes. *Alcohol* **1987**, *1*, 219-223.

[95] Grafstrom, R.C.; Curren, R.D.; Yang, L.L.; Harris, C.C. Aldehyde-induced inhibition of DNA repair and potentiation of N-nitrosocompound-induced mutagenesis in cultured human cells. *Prog. Clin. Boil. Res.* **1986**, *209A*, 255-264.

[96] Feron, V.J.; Kuper, C.F.; Spit, B.J.; Renzei, P.G.; Woutersen, R.A. Glass fibers and vapor phase components of cigarette smoke as cofactors in experimental respiratory tract carcinogenesis. *Carcinog. Comp. Surv.* **1985**, *8*, 93-118.

[97] Esmat, A.; El-Demerdash, E.; El-Mesallamy, H.; Abdel-Naim, A.B. Toxicity and oxidative stress of acrylonitrile in rat primary glial cells: preventive effects of N-acetylcysteine. *Toxicol. Lett.* **2007**, *171*, 111-118.

[98] Marsh, B.G.; Youk, A.O.; Collins, J.J. Reevaluation of lung cancer risk in the acrylonitrile cohort study of the National Cancer Institute and the National Institute for Occupational Safety and Health. *Scand. J. Work Environ. Health* **2001**, *27*, 5-13.

[99] Sakurai, H. Carcinogenicity and other health effects of acrylonitrile with reference to occupational exposure limit. *Ind. Health* **2000**, *38*, 165-180.

[100] Collins, J.J.; Acquavella, J.V. Review and meta-analysis of studies of acrylonitrile workers. *Scand. J. Work Environ. Health*. **1998**, *24*(suppl), 71-80.

[101] IARC International Agency for Research on Cancer. Re-evaluation of some organic chemicals. Hydrazine and Hydrogen peroxide. In: *IARC Monographs on Evaluation of Carcinogenic Risks to Humans.* Lyon, **1999**, Vol. *71*, pp. 43-108.

[102] Zhang, L.M.; Kamendulis, L.M.; Jiang, J.; Xu, Y.; Klauning, J.E. Acrylonitrile-induced morphological transformation in Syrian hamster embryo cells. *Carcinogenesis* **2000**, *21*, 727-733.

[103] Keusler, T.W.; Thrush, M.A. Role of oxygen radicals in tumor promotion. *Environ. Mutagen*. **1986**, *6*, 593-616.

[104] Ardelt, B.K.; Borowitz, J.L.; Isom, G.E. Brain lipid peroxidation and antioxidant protectant mechanisms following acute cyanide intoxication. *Toxicology* **1989**, *56*, 147-154.

[105] Sponsiello-Wang, Z.; Sanders, E.; Weitkunant, R. Occupational acrylonitrile exposure and lung cancer: a meta-analysis. *J. Environ. Sci. Health C. Environ. Carcinog. Ecotoxicol. Rev.* **2006**, *24*, 257-284.

[106] Wynder, E.L.; Hoffman, D. Tobacco and Tobacco Smoke. *Studies in Experimental Carcinogenesis.* New York: Academic Press, **1967**, p. 450.

[107] Van Bladeren, L.P.; Delbressine, J.J.; Hoogeterp, J.J.; Beaumont, A.H.; Breimer, D.D.; Seutter-Berlage, F.; van der Gen, A. Formation of mercapturic acids from acrylonitrile, crotononitrile, and cinnamonitrile by direct conjugation and *via* an intermediate oxidation process. *Drug. Metabol. Dispos.* **1981**, *9*, 246-249.

[108] Geiger, L.E.; Hoby, L.L.; Guengerich, F.P. Metabolism of acrylonitrile by isolated rat hepatocytes. *Cancer Res.* **1983**, *43*, 3080-3087.

[109] Lambotte-Vanderpaer, M.; Duverger-Van Bogaert, M.; Rollman, B. Metabolism and mutagenicity of acrylonitrile: an *in vivo* study. *Mutagenesis* **1985**, *7*, 655-662.

[110] Guengerich, F.P.; Kim, D.H.; Iwasaki, M. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol.* **1991**, *4*, 168-179.

[111] Guengerich, F.P.; Geiger, L.E.; Hogy, L.L. Wright, P.L. *In vitro* metabolism of acrylonitrile to 2-cyanoethylene oxide, reaction with glutathione, and irreversible binding to proteins and nucleic acids. *Cancer Res.* **1981**, *41*, 4925.

[112] Roberts, A.E.; Lacy, S.A.; Pilon, D.; Turner, M.J. Jr; Ricker,t DE. Metabolism of acrylonitrile to 2-cyanoethylene oxide in F-344 rat liver microsomes, lung microsomes, and lung cells. *Drug Metab. Dispos.* **1989**, *17*, 481-486.

[113] Zitting, A.; Heinonen, T. Decrease of reduced glutathione in isolated rat hepatocytes caused by acrolein, acrylonitrile, and the thermal degradation products of styrene copolymers. *Toxicology* **1980**, *17*, 333-341.

[114] Ronto, G.; Grof, P.; Buisson, J.P.; Einhorn, J.; Demerseman, P. Genotoxic effectivity--comparison of 36 nitrated furan and arenofuran derivatives on a quantitative scale. Statistical comparison of T7 and other short-term tests. *Mutagenesis* **1992**, *7*, 243-249.

[115] Lee, H.S.; Bian, S.; Chen, Y.L. Genotoxicity of 1,3-dithiane and 1,4-dithiane in the CHO/SCE assay and the Salmonella/microsomal test. *Mutat. Res.* **1994**, *321*, 213-218.

[116] Chen, L.; Hecht, S.S.; Peterson, A.L. Identification of cis-2-butene-1,4-dial as a microsomal metabolite of furan. *Chem. Res. Toxicol.* **1995**, *8*, 903-906.

[117] Kedderis, G.L.; Carfagua, M.A.; Held, S.D.; Batra, R.; Murphy, J.E.; Gargas, M.L. Kinetic analysis of furan biotransformation by F-344 rats *in vivo* and *in vitro. Toxicol. Appl. Pharmacol.* **1993**,*123*, 274-282.

[118] NTP. Toxicology and carcinogenesis studies of Furan (CAS No 110-06-9) in F34h Rats and B6C3F Mice (Cavage studies). Technical Report Series No 402. NIH Publications No 93-2857. Research Triangle Park, NC: National toxicology Program **1993**, p. 2861.

[119] Faber, E. Chemical carcinogenesis: a biologic perspective. *Am. J. Pathol.* **1982**, *106*, 271.

[120] Mason, R.P.; Harrelson, W.E.; Kalyanaraman, B.; Motley, C.; Peterson, F.J.; Holtzman, J.L. *Free Radical Metabolites of Chemical Carcinogens in Free Radicals, Lipid Peroxidation and Cancer*, Mc Brien, D.C.H.; Slater, T.F. Eds. Academic Press: London, **1982**, p. 377.

[121] Stevenson, M.A.; Pollock, S.S.; Coleman, C.N.; Calderwood, S.K. X-irradiation, phorbol esters, and H2O2 stimulate mitogen-activated protein kinase activity in NIH-3T3 cells through the formation of reactive oxygen intermediates. *Cancer Res.* **1994**, *54*, 12-15.

[122] Guyton, K.Z.; Liu, Y.; Gorospe, M.; Xu, Q.; Holbrook, N. Activation of mitogen-activated protein kinase by H2O2. Roll in cell survival following oxidant injury. *J. Biol. Chem.* **1994**, *271*, 4138-4142.

[123] Abe, M.K.; Kartha, S.; Korpova, A.Y.; Li, J.; Liu, P.T.; Kuo, W.L.; Hershenson, M.B. Hydrogen peroxide activates extracellular signal-regulated kinase *via* protein kinase C, Raf-1, and MEK1. *Am. J. Respir. Cell. Mol.Biol.* **1998**, *18*, 562-569.

[124] De Wit, R.; Boonstra, J.; Verklei, A.J.; Post, J.A. Large Scale Screening Assay for the Phosphorylation of Mitogen-Activated Protein Kinase in Cells. *J. Biomol. Screen.* **1998**, *3*, 277-284.

[125] Rao, G.N. Hydrogen peroxide induces complex formation of SHC-Grb2-SOS with receptor tyrosine kinase and activates Ras and extracellular signal-regulated protein kinases group of mitogen-activated protein kinases. *Oncogene*, **1996**, *13*, 713-719.

[126] Gonzalez-Rubio, M.; Voit, S.; Rodriguez-Puyol, D.; weber, M.; Mary, M. Oxidative stress induces tyrosine phosphorylation of PDGF alpha-and beta-receptors and pp60c-src in mesangial cells. *Kidney Int.* **1996**, *50*, 164-173.

[127] Gamon, S.; Shimizu, N. Hydrogen peroxide preferentially enhances the tyrosine phosphorylation of epidermal growth factor receptor. *FEBS Lett.* **1995**, *357*, 161-164.

[128] Knebel, A.; Rahmsdorf, H.J.; Ulrich, A.; Herrlich, P. Dephosphorylation of receptor tyrosine kinases as target of regulation by radiation, oxidants or alkylating agents. *EMBO J.* **1996**, *15*, 5314-5325.

[129] Rosette, C.; Karin, M. Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* **1996**, *274*, 1194-1197.

[130] Lee, S.R.; Kwou, K.S.; Kim, S-R.; Rhee, S.G. Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. *J. Biol. Chem.* **1996**, *273*,15366-15372.

[131] Hunter, T. Oncoprotein networks. *Cell* **1997**, *88*, 333-346.

[132] Davis, R.J. The mitogen-activated protein kinase signal transduction pathway. *J. Biol. Chem.* **1993**, *268*, 14553-14556.

[133] Boorstein, R.J.; Cadet, J. The repairability of oxidative free radical mediated damage to DNA: a review. *Int. J. Ratiat. Biol.* **1988**, *54*, 131-150.

[134] Halliwell, B.; Amoma, O.I. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett.* **1991**, *281*, 9-19.

[135] Goldstein, B.D.; Witz, G. Free radicals and carcinogenesis. *Free Radic. Res. Commun.* **1990**, *11*, 3.

[136] Kensler, T.W.; Egner, P.A.; Taffe, B.G.; Trush, M.A. *Role of Free Radicals in Tumor Promotion and Progression in Skin Carcinogenesis: Mechanisms and Human Relevance*. Alan, R. Ed.; Liss: New York, **1989**, p. 233.

[137] Cerruti, P.A. Prooxidant states and tumor promotion. *Science* **1985**, *277*, 375.


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