Trends Article – Minireview

Common Solvent Toxicity: Autoxidation of Respiratory Redox-Cyclers Enforced by Membrane Derangement

Thomas R. Garbe and Hideaki Yukawa*

Research Institute of Innovative Technology for the Earth (RITE), 9–2, Kizugawadai, Kizu-cho, Soraku-gun, Kyoto, 619–0292 Japan.
Fax: +81 774 75 2321. E-mail address: yukawa@rite.or.jp

* Author for correspondence and reprint requests

Z. Naturforsch. **56 c,** 483–491 (2001); received February 13/March 16, 2001

Respiration, Lipophilicity, Prooxidants

Unspecific biological effects of chemically diverse solvents strikingly reveal the unifying motif of oxidant toxicity both in higher organisms and in aerobic bacteria. In a few spectacular cases, solvent metabolites with oxidant properties were demonstrated, which however cannot explain extrahepatic toxicity, e.g. in muscle and nerve cells. A common source of solvent-inducible oxidants, by contrast, is suggested to be located in mitochondria or, more general, in membranes where the respiratory chain operates. Orderly respiration depends on membrane integrity, which is invariably compromised by exposure to most solvents and many other lipophils. In rat mitochondria, toluene-induced membrane derangement has been directly implicated with superoxide production, resulting from autoxidation of the membrane-located respiratory redox-cycler ubisemiquinone. A related mechanism may occur in bacteria: Exposure of *Escherichia coli* to lipophils such as ethanol, tetralin, indole, chlorpromazine and procaine, or to heat shock, induces anti-oxidant proteins, which are reliable indicators of increased oxidant levels. Although many molecular details remain to be elucidated, this review documents that oxidant toxicity of lipophilic compounds is a common physiological phenomenon correlated with derangement of membranes where respiratory processes take place. Subjective consequences of acute oxidant injury are probably the hangover from alcohol and nicotine consumption, and the sudden death from recreational solvent abuse. Suggestions concerning oxidants as major contributors to ageing remain unchallenged.

Introduction

Current reports on the unspecific toxicity of solvents and solid lipophils reiterate the dominance of oxidant effects (Table I), which occur in aerobic cells of both eukaryotes and prokaryotes, irrespective of their function or organisation. Such uniform toxicity suggests a common mechanism, which is however incompatible with the ruling theory that links solvent oxidant toxicity exclusively to metabolic activation. The data presented in this review support the view throughout that lipophils induce autoxidation of natural reductants from the respiratory chain. Autoxidation generates the reduced oxygen derivative superoxide ($O_2^{-\cdot}$) (Eqn. (1)), and consequently hydroperoxyl radical ($HO_2^{\cdot}$), hydrogen peroxide (H_2O_2) (Eqn. (2)), and hydroxyl radical ($HO^{\cdot}$) (Eqn. (3)) (Fridovich, 1999).

$$O_2 + e^- \longrightarrow O_2^{-\cdot} \quad (1)$$

$$2\,O_2^{-\cdot} \xrightarrow{2H^+} HOOH + O_2 \quad (2)$$

$$HOOH + Fe^{2+} \longrightarrow HO^{\cdot} + HO^- + Fe^{3+} \quad (3)$$

The natural reductants from the respiratory chain include the ubiquinone redox-cycling system with its reduced structures from one and two electron transfer, ubisemiquinone and ubiquinol, which reside inside lipid bilayer membranes, where the compounds are freely diffusible, but protected from water (Nohl and Stolze, 1992). Exposure of mitochondria to toluene leads to accumulation of toluene in the membrane, causing derangement of the molecular order of the phospholipid bilayer, and increased membrane fluidity. A decrease of extramitochondrial proton accumulation suggests in-

0939–5075/2001/0700–0483 $ 06.00

Table I. Physiological effects of lipophilic stimulants

Stimulant	Physiological and toxic effects
Hydrocarbons	• accumulation of hydrocarbons in membranes between fatty acid residues and between opposing monolayers (Sikkema *et al.*, 1995) • membrane toxicity in microorganisms (Sikkema *et al.*, 1995) • sudden death following recreational inhalation of propane, *n*-butane or isobutane (Rohrig, 1997)
Benzene	• generation of hydroxyl radical in fresh meat (Karam and Simic, 1989)
Toluene	• euphoria, sudden death, injury to liver, kidneys and brain (Flanagan and Ives, 1994) • superoxide by autoxidation of ubisemiquinone in isolated mitochondria (Nohl *et al.*, 1996)
Tetraline	• membrane derangement and proton penetration (Sikkema *et al.*, 1992) • enhanced alkylhydroperoxide reductase subunit C (AhpC) activity in resistant *Escherichia coli* (Ferrante *et al.*, 1995)
Indole	• hemolysis in ponies (Paradis *et al.*, 1991) • induction of AhpC in *E. coli* K12 and overproduction of AhpC in indole-resistant *E. coli* variant (Garbe *et al.*, 2000a)
Tobacco smoke	• generates active oxygen and superoxide (Kodama *et al.*, 1997)
Chlorpromazine, Procaine	• induction of superoxide dismutase in *E. coli* (Zhang and Yonei, 1991)
Hexachloro-cyclohexane (HCH)	• membrane derangement and lysis of human erythrocytes (Verma and Singhal, 1991) • lipid peroxidation in rat testes (Samanta *et al.*, 1999)
Polychlorinated biphenyls (PCBs)	• neuroactive PCBs disruptive of mitochondrial oxidative energy production (Maier *et al.*, 1994) • neuroactive PCBs produced oxidants in rat synaptosomes (Voie and Fonnum, 2000)
Halogenated alkanes	• euphoria, sudden death, injury to liver, kidneys and brain (Flanagan and Ives, 1994) • vasodilation, cardiac arrest (sudden death), hemolytic anemia (reviewed by Tse *et al.*, 1990) • lipid peroxidation (Channel *et al.*, 1998, Toraason *et al.*, 1999, Plaa, 2000) • oxidative DNA damage (Toraason *et al.* 1999) • alteration of mitochondrial structure by chloroform (Guastadisegni *et al.*, 1999) • generation of hydroxyl radical in white and red muscle meat (Karam and Simic, 1990)
Alkyl nitrites (Poppers)	• euphoria, nitric oxide release causative of smooth muscle relaxation and N-nitrosamine formation, methemoglobinemia (Maikel, 1988; Hecht, 1997)
Nitro propane	• lipid peroxidation in liver, lung and kidney of rats (Kim *et al.*, 1998)
Methanol	• alcohol dehydrogenase-dependent oxidation to formic acid causative of acidosis and ocular toxicity
Ethanol	• interacts with lipid membranes at the hydrophilic head, impairs the membrane-water interaction and destabilises the membrane (Ueda and Yoshida, 1999) • increases saturation of mitochondrial fatty acids (Rubin and Rottenberg, 1982) • derangement of yeast membrane is followed by proton penetration (Cartwright *et al.*, 1986, Leáo *et al.*, 1984) • activates the Oxy^R regulator in *E. coli* (Belkin *et al.*, 1996) and in *Salmonella* (Morgan *et al.*, 1986) • induction of cytoplasmic catalase and mitochondrial Mn-superoxide dismutase in yeast (Piper, 1995) • oxidants in liver, pancreatic, brain and testicular tissues of the rat (Mantle and Preedy, 1999) • oxidants in a gastric mucosal cell line (Hirokawa *et al.*, 1998) • superoxide induction and lipid peroxidation in mitochondria (Kurose *et al.*, 1996)

Table I. (cont.)

Stimulant	Physiological and toxic effects
Ethanol, cont.	• hydroxyl radical in white and red muscle meat suggested to be due to autoxidation of ubisemiquinone (Karam and Simic, 1990) • oxidants suggested to cause headache, hangover, stroke and sudden death from binge drinking (≥80 g/day) (Altura and Altura, 1999) • promotes hepatic mitochondrial DNA deletions in alcoholic patients (Mansouri *et al.*, 1997; Kurose *et al.*, 1996)
n-Butanol	• membrane toxicity in *Clostridium* (Bowles and Ellefson, 1985)
Heat shock	• unfolds proteins and fluidises membranes due to increased molecular motion (Ueda and Yoshida, 1999) • alteration of fatty acid profile in *E. coli* (Mejia *et al.*, 1999) • induction of Mn-superoxide dismutase in *Salmonella* (Lee *et al.*, 1983) and *E. coli* (Privalle and Fridovich, 1987) • induction of anti-oxidant proteins in yeast (Piper, 1995; Davidson *et al.*, 1996)

creased membrane permeablity (Nohl *et al.*, 1996), which allows contact between ubisemiquinone and the water phase, causing autoxidation and release of superoxide (Nohl *et al.*, 1996; 1998).

The toluene-enforced autoxidation of ubisemiquinone is being generalized here as the prototypic mechanism for superoxide generation by lipophils and heat shock in mammalian cells (Table I). Heat shock, i.e. a sudden elevation of temperature, increases membrane fluidity (Ueda and Yoshida, 1999) and thus affects respiration.

Generation of oxidants in bacteria by exposure to lipophils has only recently become known to be a general phenomenon (Table I), but to date no mechanistic studies have been carried out. It is known, however, that interruption of the electron flow, either by the respiratory inhibitor cyanide or by the lack of ubiquinone, generates oxidants in bacteria (Matsushita *et al.*, 1998; Messner and Imlay, 1999; Søballe and Poole, 2000). This principle, which relies on autoxidation of accumulated FADH cofactor of NADH dehydrogenase II (Messner and Imlay, 1999), may also pertain to lipophil-induced membrane derangement. In either case, membrane derangement is the likely primary event in lipophil-mediated generation of oxidants.

Solvent oxidant toxicity via metabolites

Common sense locates solvent toxicity in the liver, where the bulk of foreign compounds is being metabolized. This view coheres with the focus of current scientific endeavour being on metabolic activation, even though the "putative chemical species in most cases ... has not necessarily been identified" (Plaa, 2000).

The theory's gold standard is based on carbon tetrachloride (CCl_4), which is enzymatically reduced by the inducible cytochrome P450 2E1 to the trichloromethyl radical ($^{\cdot}CCl_3$). Its rapid autoxidation generates the trichloromethylperoxyl radical ($^{\cdot}OOCCl_3$), which initiates lipid peroxidation (Fridovich, 1999; Cheeseman *et al.*, 1985; Gutteridge and Halliwell, 1990). Additionally, the trichloromethyl radicals also bind covalently to lipids, proteins and nucleic acids, which probably accounts for the extraordinary toxicity of carbon tetrachloride. Its toxicity is estimated to be up to 20 times higher than that of chloroform or of other halogenated alkanes, whose metabolization does not generate radicals (Plaa, 2000; Ruch *et al.*, 1986). Thus, carbon tetrachloride toxicity poses a specific case, because of which it cannot provide a model for the general oxidant toxicity of solvents.

Alcohols give analogous peroxyl radicals from cytochrome P450 2E1-mediated metabolic activation, but this is an accidental side reaction. The enzyme's function relies, like that of the constitutive alcohol dehydrogenase, on the oxidation of alcohols to the corresponding aldehyde, but happens at the expense, rather than gain, of NAD(P)H.

Acute intragastric dosing of rats with 7 g/kg methanol gave the hydroxymethyl radical ($^{\cdot}CH_2$–OH) (Kadiiska and Mason, 2000). The excessive dose indicates radical formation as a minor event. Higher yields of peroxyl radicals from alcohols emerge from chronic alcoholism, reflecting the de-

pendence on enzyme induction. In chronically ethanol-fed rats, where cytochrome P450 2E1 is induced, the α-hydroxyethyl radical ($^{\cdot}CH_2-CH_2-OH$) has been suggested as the dominant initial metabolic product (Kurose *et al.*, 1996). However, the α-hydroxyethyl radical cannot explain extrahepatic oxidant toxicity, such as involved in gastric mucosal cell death (Hirokawa *et al.*, 1998), in cardiovascular or in brain injury (Altura and Altura, 1999). One undoubted benefit of enzyme induction is the accelerated elimination of ethanol (Lieber, 1997). Teleological doctrine suggests that alleviation of acute-type ethanol toxicity outweighs the drawbacks of enzyme-dependent radical formation.

Oxidant toxicity of hydrocarbons and xenobiotics

By providing evidence that toluene, an inert aromatic hydrocarbon, causes generation of superoxide in isolated mitochondria from rat heart, Nohl and coworkers (1996) have demonstrated a novel principle of oxidant toxicity. An earlier version of this mechanism was used to explain benzene oxidant toxicity, which was concluded from the generation of *o*-hydroxyphenylalanine (*o*-tyrosine) in fresh chicken breast meat. The amount of *o*-tyrosine created by hydroxylation of phenylalanine in meat after exposure to benzene for 1 h (50 g meat per 25 ml benzene) was equivalent to a ^{60}Co-γ-radiation dose of 3 kGy (Karam and Simic, 1989).

Resistance of *Escherichia coli*, both to 1,2,3,4-tetrahydronaphthaline (tetralin) and to indole, is linked to constitutively increased activity of alkylhydroperoxide reductase subunit C (AhpC) (Ferrante *et al.*, 1995; Garbe *et al.*, 2000a). AhpC is involved in the reduction of hydroperoxides to the corresponding alcohols, which relieves the cell of numerous toxicities including initiation of radical chain reactions in membranes (Gutteridge and Halliwell, 1990). Commonly, AhpC is under positive control of OxyR, a regulator of defences against hydrogen peroxide (Storz and Imlay, 1999). Increased levels of AhpC are thus an indicator of oxidant toxicity.

The unexpected involvement of AhpC in resistance to tetralin was presumed to be mediated by tetralin hydroperoxides, however, no such compounds were demonstrated. Similarly, no indole hydroperoxides were found in indole-supplemented bacterial supernatants, despite thorough chromatographic analyses, which identified bisindolyl carboxylic acids as the only bio-transformed indoles (Garbe *et al.*, 2000b). Hence, endogenous oxidants, as discussed in the introduction, must be considered.

The tranquilizer chlorpromazine (2-chloro-N,N-dimethyl-10H-phenothiazine-10-propanamine) and the local anaesthetic procaine (4-aminobenzoic acid 2-(diethylamino)-ethyl ester) both induce superoxide dismutase in *E. coli* (Zhang and Yonei 1991). The enzyme catalyses the disproportionation of superoxide as shown in Eqn. (2), which provides an important means of anti-oxidant defence in bacteria and higher organisms (Fridovich 1995). Induction of superoxide dismutase by both neuroactive compounds is a valid demonstration for the presence of superoxide. It converges with the enzyme's induction by heat shock (Table I). In both cases the generation of superoxide was considered to be due to "disruption of the electron transport assemblies of the plasma membrane" (Privalle and Fridovich, 1987).

The number of proven prooxidant organic compounds is far exceeded by the number of lipophils which exert unspecific microbiocidal activity. It has been suggested that unspecific microbiocidal activity of lipophils is based on interactions with membranes (Sikkema *et al.*, 1995). Based on recent results which demonstrated prooxidant activity of structurally unrelated lipophils in *E. coli*, it is suggested that microbiocidal activity of lipophils may be exacerbated by superoxide, generated by autoxidation of respiratory reductants following membrane derangement. Organic compounds with unspecific microbiocidal activity include fatty acids (Khulusi *et al.*, 1995), local and general anesthetics, antimalarial agents, the anti-gout drug probenecid (4-[(dipropylamino)sulfonyl]benzoic acid), antihistamines, barbiturates, salicylates, diuretics, steroids, phenols, the anti-oxidants BHT and BHA (butylated hydroxytoluene and butylated hydroxyanisole), and other organic compounds (Sikkema *et al.*, 1995).

The mechanism may also dominate the oxidant toxicity of hexachlorocyclohexane (HCH) and of polychlorinated biphenyls (PCBs) in mammals. The insecticide HCH was shown to cause membrane derangement, hemolysis (Verma and Sin-

ghal, 1991) and lipid peroxidation in rat testes (Samanta *et al.*, 1999). Neuroactive PCBs were reported to generate oxidants in nerve cells (Voie and Fonnum, 2000).

Toxicity of halogenated alkanes

Hepatotoxicity of chlorinated hydrocarbons is acknowledged to predominantly rely on lipid peroxidation. In case of carbon tetrachloride, the causation of lipid peroxidation is dominated by the reactive trichloromethylperoxyl radical ($^{\cdot}OOCCl_3$), which arises by metabolic activation as described.

Carbon tetrachloride also causes oxidants independent of metabolic activation. This was demonstrated by the generation of the hydroxyl radical adduct *o*-tyrosine in fresh muscle meat from chicken or beef. The hydroxyl radical was suggested to originate from autoxidation of ubisemiquinone via superoxide (Karam and Simic, 1990).

Cardiovascular oxidant toxicity of carbon tetrachloride also fails explanation by bioactivation, since the solvent caused oxidant toxicity in cultured arterial endothelial and aortic smooth muscle cells to similar degrees as did dichloro- and trichloroethane (Tse *et al.*, 1990). Hepatic microsomal activation of the latter solvents is 1000 times slower than that of carbon tetrachloride.

Extrahepatic oxidant toxicity independent of bioactivation is also indicated in trichloroethylene poisoning (Tse *et al.*, 1990). It exerts prominent vasodilation, which is a property shared with superoxide and hydroxyl radical. Moreover, hydroxyl radical causes inflammation of blood vessels (vasculitis), a condition associated with chronic solvent abuse (Tse *et al.*, 1990).

Chloroform- ($CHCl_3$) mediated oxidant hepatotoxicity is unrelated to peroxy radical formation, since no such products have been detected (Knecht and Mason, 1991). Instead, the cytochrome P450 2E1-dependent oxidation of chloroform in phenobarbital-pretreated rats yields phosgene ($Cl_2C{=}O$), a thiol-depleting alkylating agent (Cheeseman *et al.*, 1985, Plaa, 2000), but its significance remains undetermined. Recently, morphologic alterations of mitochondria from chloroform-treated rats have been demonstrated, which suggest mitochondrial involvement in chloroform-induced hepatotoxicity (Guastadisegni *et al.*, 1999).

Potentiation of liver injury by halogenated solvents has been studied extensively. It was found that the simultaneous or sequential combination of an alcohol or a ketone with certain halogenated solvents was unexpectedly toxic (Plaa, 2000). Exacerbation of toxicity by ketone potentiation pertains to the following solvents: carbon tetrachloride, chloroform, 1,1,2-trichloroethane, dichlorethylene, bromoform, bromodichloromethane and dibromochloromethane. By contrast, weak hepatotoxic chlorinated alkanes evade potentiation by ketones. Those include 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, trichloroethylene and tetrachloroethylene (Plaa, 2000). Speculations concerning the mechanism of potentiation focus on synergistic action of different solvents that act as enzyme inductors, but no mechanism was suggested. The membrane-derangement model, however, provides a plausible answer: Solvents of different polarity (lipophilicity) act on a membrane at different sites. The water-immiscible halogenated alkanes are less polar than the lower alcohols or ketones, and have been found to penetrate membranes to the second CH_2-layer from the water-micelle interface (Ueda and Yoshida, 1999). The addition of a different quality of membrane interaction should exert a stronger effect on membrane order than more of the same agent, resulting in earlier superoxide production from autoxidizing respiratory reductants.

Alcohol toxicity

Lower monovalent alcohols are polar solvents and do not, by contrast to hydrocarbons, insert into phospholipid membranes, but interact with the hydrophilic head. Since membranes are supported by the hydrogen-bonded water matrix, the reduced membrane-water interaction results in membrane destabilization and disorder (Ueda and Yoshida, 1999). In contrast to hydrocarbons, alcohols decrease membrane fluidity (Ueda and Yoshida, 1999). Nevertheless, ethanol treatment of yeast resulted in membrane penetrability for protons (Leáo and van Uden, 1984; Cartwright *et al.*, 1986), which is the precise condition for superoxide generation by autoxidizing ubisemiquinone (Nohl *et al.*, 1996; 1998).

Further evidence for the involvement of membranes in alcohol toxicity comes from multiple re-

ports on changes of lipid composition as a cellular adaptation to alcohol. In *E. coli*, adaptation to ethanol coincided with an increase of unsaturated fatty acid residues in lipids (Ingram *et al.*, 1980). Likewise, *Saccharomyces cerevisiae* increased the amount of unsaturated fatty acid residues in lipids in response to ethanol exposure (Kajiwara *et al.*, 1996). In *Drosophila* larvae, dietary ethanol caused the loss of long-chain fatty acids in membrane lipids, which decreased ethanol tolerance. Larvae of an ethanol-tolerant strain maintained the long-chain fatty acids upon dietary ethanol (Miller *et al.*, 1993). Nerve cell lipids from chicken embryos also underwent changes of composition upon dietary ethanol exposure (Miller *et al.*, 2000).

Chronic ethanol consumption by mammals and humans alters the fatty acid composition of phospholipids in liver and heart mitochondria (Gordon, 1984; Foudin *et al.*, 1986; Cunningham and Spach, 1987; Zidenberg-Cherr *et al.*, 1991). The change of lipid composition may increase membrane resistance toward ethanol-induced derangement and peroxidation (Rubin and Rottenberg, 1982; Zidenberg-Cherr *et al.*, 1991). Altered lipid composition in erythrocytes from chronic alcohol patients had a lower docosahexaenoic acid content, which was suggested to account for decreased sensitivity to lipid peroxidation (Gatti *et al.*, 1993).

Exposure to alcohol causes a modulation of protein synthesis also. In *Salmonella typhimurium* and in *E. coli,* ethanol activates the OxyR regulator, which is also activated by hydrogen peroxide, organic hydroperoxides and redox-cyclers (Morgan *et al.*, 1986; Belkin *et al.*, 1996). Activated OxyR enables the transcription of nine genes, which encode for products such as catalase, AhpC/F and glutathione reductase, which are involved in defence of oxidant toxicity (Storz and Imlay, 1999). Thus, ethanol exerts oxidant toxicity in these bacteria. Yeast, upon exposure to ethanol, increased activities of cytoplasmic catalase and Mn superoxide dismutase. A yeast mutant lacking the latter activity proved unusually sensitive to ethanol (Piper, 1995). None of the microbial anti-oxidant responses which follow exposure to ethanol appear dependent on metabolic activation.

In rodents and man, acute alcohol oxidant toxicity appears likewise independent of metabolic activation (Kurose *et al.*, 1996), and arises in mitochondria, rather than in microsomes (Nordmann *et al.*, 1992; Kukielka *et al.*, 1994; Wieland and Lauterburg, 1995; Kurose *et al.*, 1996; Hirokawa *et al.*, 1998). Oxidative damage to mitochondrial DNA and dramatic losses of mitochondrial DNA from liver cells of alcoholic rat models and alcoholic patients implicate respiratory activities in chronic ethanol toxicity (Mansouri *et al.*, 1997; Cahill *et al.*, 1999). Ethanol-associated damage to mitochondria is further apparent by structural lesions and decreased respiration, resulting in fatty liver (Pessayre *et al.*, 1999). Suggestions on the underlying mechanism have sparsely been made. However, with respect to ethanol-induced *o*-tyrosine generation in fresh meat, Karam and Simic (1990) have implicated autoxidizing ubisemiquinone in "stressed" mitochondria. The conclusion that this mechanism is responsible for the other mentioned mitochondrial injuries is inescapable. It suggests that excessive acute or chronic ethanol ingestion is particularly damaging.

The mechanism may also be responsible for cellular losses of Mg^{2+} (Altura and Altura, 1999), which have been implicated with "mitochondrial permeability transition", a phenomenon inducible by exposure to diverse oxidants including superoxide (Vercesi *et al.*, 1997). Cellular losses of Mg^{2+} promote hypoxia (Altura and Altura, 1999), which has previously been reported as another effect of ethanol intoxication (Hijioka *et al.*, 1993; Thurman *et al.*, 1999). The vicious circle closes by the fact that hypoxia stimulates autoxidation of ubisemiquinone in mitochondria (Nohl *et al.*, 1996).

Altura B. M. and Altura B. T. (1999), Association of alcohol in brain injury, headaches, and stroke with brain-tissue and serum levels of ionized magnesium: a review of recent findings and mechanisms of action. Alcohol. **19**, 119–130.

Belkin S., Smulski D. R., Vollmer A. C., Van Dyk T. K. and LaRossa R. A. (1996), Oxidative stress detection with *Escherichia coli* harboring a katG'::lux fusion. Appl. Environ. Microbiol. **62**, 2252–2256.

Bowles L. K. and Ellefson W. L. (1985), Effects of butanol on *Clostridium acetobutylicum*. Appl. Environ. Microbiol. **50**, 1165–1170.

Cahill A., Stabley G. J., Wang X. and Hoek J. B. (1999), Chronic ethanol consumption causes alterations in the structural integrity of mitochondrial DNA in aged rats. Hepatology **30**, 881–888.

Carlsen H. N., Degu H. and Lloyd D. (1991), Effects of alcohols on the respiration and fermentation of aerated suspensions of baker's yeast. J. Gen. Microbiol. **137**, 2879–3083.

Cartwright C. P., Juroszek J.-R., Beavan M. J., Ruby F. M. S., De Morais S. M. F., Rose A. H. (1986), Ethanol dissipates the proton-motive force across the plasma membrane of *Saccharomyces cerevisiae*. J. Gen. Microbiol. **132**, 369–377.

Cheeseman K. H., Albano E. F., Tomasi A. and Slater T. F. (1985), Biochemical studies on the metabolic activation of halogenated alkanes. Environ. Health Perspect. **64**, 85–101.

Cunningham C. C. and Spach P. I. (1987), The effect of chronic ethanol consumption on the lipids in liver mitochondria. Ann. N. Y. Acad. Sci. **492**, 181–192.

Davidson J. F., Whyte B., Bissinger P. H. and Schiestl R. H. (1996), Oxidative stress is involved in heat-induced cell death in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **93**, 5116–5121.

Ferrante A. A., Augliera J., Lewis K. and Klibanov A. M. (1995), Cloning of an organic solvent-resistance gene in *Escherichia coli*: the unexpected role of alkylhydroperoxide reductase. Proc. Natl. Acad. Sci. **92**, 7617–7621.

Flanagan R. J. and Ives R. J. (1994), Volatile substance abuse. Bull. Narc. **46**, 49–78.

Fridovich I. (1995), Superoxide radical and superoxide dismutases. Annu. Rev. Biochem. **64**, 97–112.

Fridovich I. (1999), Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? Ann. N. Y. Acad. Sci. **893**, 13–18.

Foudin L., Sun G. Y. and Sun A. Y. (1986), Changes in lipid composition of rat heart mitochondria after chronic ethanol administration. Alcohol. Clin. Exp. Res. **10**, 606–609.

Garbe T. R., Kobayashi M. and Yukawa H (2000a), Indole-inducible proteins in bacteria suggest membrane and oxidant toxicity. Arch. Microbiol. **173**, 78–82.

Garbe T. R., Kobayashi M., Shimizu N., Takesue N., Ozawa M. and Yukawa H. (2000b), Indolyl carboxylic acids by condensation of indoles with α-keto acids. J. Nat. Prod. **63**, 596–598.

Gatti P., Viani P., Cervato G., Testolin G., Simonetti P. and Cestaro B. (1993), Effects of alcohol abuse: studies on human erythrocyte susceptibility to lipid peroxidation. Biochem. Mol. Biol. Int. **30**, 807–817.

Gordon E. R. (1984), Alcohol-induced mitochondrial changes in the liver. Recent Dev. Alcohol. **2**, 143–158.

Guastadisegni C., Balduzzi M., Mancuso M. T. and Di Consiglio E. (1999), Liver mitochondria alterations in chloroform-treated Sprague-Dawley rats. J. Toxicol. Environ. Health **57**, 415–429.

Gutteridge J. M. C. and Halliwell B. (1990), The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem. Sci. **15**, 129–135.

Hecht S. S. (1997), Approaches to cancer prevention based on an understanding of N-nitrosamine carcinogenesis. Proc. Soc. Exp. Biol. Med. **216**, 181–191.

Hijioka T., Sato N., Matsumura T., Yoshihara H., Takei Y., Fukui H., Oshita M., Kawano S. and Kamada T. (1993), Ethanol-induced disturbance of hepatic microcirculation and hepatic hypoxia. Biochem. Pharmacol. **41**, 1551–1557.

Hirokawa M., Miura S., Yoshida H., Kurose I., Shigematsu T., Hokari R., Higuchi H., Watanabe N., Yokoyama Y., Kimura H., Kato S. and Ishii H. (1998), Oxidative stress and mitochondrial damage precedes gastric mucosal cell death induced by ethanol administration. Alcohol. Clin. Exp. Res. **22 (Suppl)**, 111S-114S.

Ingram L. O., Vreeland N. S. and Eaton L. C. (1980), Alcohol tolerance in *Escherichia coli*. Pharmacol. Biochem. Behav. **13 Suppl 1**, 191–195.

Kadiiska M. B. and Mason R. P. (2000), Acute methanol intoxication generates free radicals in rats: an ESR spin trapping investigation. Free Radic. Biol. Med. **28**, 1106–1114.

Kajiwara S., Shirai A., Fujii T., Toguri T., Nakamura K. and Ohtaguchi K. (1996), Polyunsaturated fatty acid biosynthesis in *Saccharomyces cerevisiae*: expression of ethanol tolerance and the FAD2 gene from *Arabidopsis thaliana*. Appl. Environ. Microbiol. **62**, 4309–4313.

Karam L. R. and Simic M. G. (1989), Mechanisms of free radical chemistry and biochemistry of benzene. Environ. Health Perspect. **82**, 37–41.

Karam L. R. and Simic M. G. (1990), Formation of ortho-tyrosine by radiation and organic solvents in chicken tissue. J. Biol. Chem. **265**, 11581–11585.

Khulusi S., Ahmed H. A., Patel P., Mendall M. A. and Northfield T. C. (1995), The effects of unsaturated fatty acids on *Helicobacter pylori* in vitro. J. Med. Microbiol. **42**, 276–282.

Kim S. J., Reiter R. J., Rouvier Garay M. V., Qi W., El-Sokkary G. H. and Tan D. X. (1998), 2-Nitropropane-induced lipid peroxidation: antitoxic effects of melatonin. Toxicology **130**, 183–90.

Knecht K. T. and Mason R. P. (1991), The detection of halocarbon-derived radical adducts in bile and liver of rats. Drug Metab. Dispos. **19**, 325–331.

Kodama M., Kaneko M., Aida M., Inoue F., Nakayama T. and Akimoto H. (1997), Free radical chemistry of cigarette smoke and its implication in human cancer. Anticancer Res. **17(1A)**, 433–437.

Kukielka E., Dicker E. and Cederbaum A. I. (1994), Increased production of reactive oxygen species by rat liver mitochondria after chronic ethanol treatment. Arch. Biochem. Biophys. **309**, 377–386.

Kurose I., Higuchi H., Kato S., Miura S. and Ishii H. (1996), Ethanol-induced oxidative stress in the liver. Alcohol. Clin. Exp. Res. 20(1 Suppl): 77A-85A.

Leáo C. and van Uden N. (1984), Effects of ethanol and other alkanols on passive proton influx in the yeast *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **774**, 43–48.

Lee P. C., Bochner B. R. and Ames B. N. (1983), AppppA, heat-shock stress, and cell oxidation. Proc. Natl. Acad. Sci. USA **80**, 7496–7500.

Lieber C. S. (1997), Cytochrome P-4502E1: its physiological and pathological role. Physiol. Rev. **77**, 517–544.

Maier W. E., Kodavanti P. R., Harry G. J. and Tilson H. A. (1994), Sensitivity of adenosine triphosphatases in different brain regions to polychlorinated biphenyl congeners. J. Appl. Toxicol. **14**, 225–229.

Maikel R. P. (1988), The fate and toxicity of butyl nitrites. In: Health Hazards of Nitrite Inhalants (Haverkos H. W., Dougherty J. A., eds.). NIDA Research Monograph 83, National Institute on Drug Abuse, Washington, DC, pp 15–27.

Mansouri A., Fromenty B., Berson A., Robin M. A., Grimbert S., Beaugrand M., Erlinger S. and Pessayre D. (1997), Multiple hepatic mitochondrial DNA deletions suggest premature oxidative aging in alcoholic patients. J. Hepatol. **27**, 96–102.

Mantle D. and Preedy V. R. (1999), Free radicals as mediators of alcohol toxicity. Adverse Drug React. Toxicol. Rev. **18**, 235–252.

Matsushita K., Yamamoto T., Toyama H. and Adacj O. (1998), NADPH oxidase system as a superoxide-generating cyanide-resistant pathway in the respiratory chain of *Corynebacterium glutamicum*. Biosci. Biotechnol. Biochem. **62**, 1968–1977.

Mejia R., Gomez-Eichelmann M. C. and Fernandez M. S. (1999), Fatty acid profile of *Escherichia coli* during the heat-shock response. Biochem. Mol. Biol. Int. **47**, 835–844.

Messner K. R. and Imlay J. A. (1999), The identification of primary sites of superoxide and hydrogen peroxide formation in the aerobic respiratory chain and sulfite reductase complex of *Escherichia coli*. J. Biol. Chem. **274**, 10119–10128.

Miller R. R. Jr., Dare A. O., Moore M. L., Kooser R. G. and Geer B. W. (1993), Long-chain fatty acids and ethanol affect the properties of membranes in *Drosophila melanogaster* larvae. Biochem. Genet. **31**, 113–131.

Miller R. R. Jr., Slathar J. R. and Luvisotto M. (2000), α-Tocopherol and γ-tocopherol attenuate ethanol-induced changes in membrane fatty acid composition in embryonic chick brains. Teratology **62**, 26–35.

Morgan R. W., Christman M. F., Jacobson F. S., Storz G. and Ames B. N. (1986), Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA **83**, 8059–8063.

Nohl H. and Stolze K. (1992), Ubisemiquinones of the mitochondrial respiratory chain do not interact with molecular oxygen. Free Rad. Res. Commun. **16**, 409–419.

Nohl H., Gille L., Schönheit K. and Liu Y. (1996), Conditions allowing redox-cycling ubisemiquinone in mitochondria to establish a direct redox couple with molecular oxygen. Free Radic. Biol. Med. **20**, 207–213.

Nohl H., Gille L. and Staniek K. (1998), The biochemical, pathophysiological, and medical aspects of ubiquinone function. Ann. N. Y. Acad. Sci. **854**, 394–409.

Nordmann R., Ribiere C. and Rouach H. (1992), Implication of free radical mechanisms in ethanol-induced cellular injury. Free Radic. Biol. Med. **12**, 219–240.

Paradis M. R., Breeze R. G., Laegreid W. W., Bayly W. M. and Counts D. F. (1991), Acute hemolytic anemia induced by oral administration of indole in ponies. Am. J. Vet. Res. **52**, 748–753.

Pessayre D., Mansouri A., Haouzi D. and Fromenty B. (1999), Hepatotoxicity due to mitochondrial dysfunction. Cell. Biol. Toxicol. **15**, 367–373.

Piper P. W. (1995), The heat shock and ethanol stress responses of yeast exhibit extensive similarity and functional overlap. FEMS Microbiol. Lett. **134**, 121–127.

Plaa G. L. (2000), Chlorinated methanes and liver injury: highlights of the past 50 years. Annu. Rev. Pharmacol. Toxicol. **40**, 43–65.

Privalle C. T. and Fridovich I. (1987), Induction of superoxide dismutase in *Escherichia coli* by heat shock. Proc. Natl. Acad. Sci. USA **84**, 2723–2726.

Rohrig T. P. (1997), Sudden death due to butane inhalation. Am. J. Forensic Med. Pathol. **18**, 299–302.

Rubin E. and Rottenberg H. (1982), Ethanol-induced injury and adaptation in biological membranes. Fed. Proc. **41**, 2465–2471.

Ruch R. J., Klaunig J. E., Schultz N. E., Askari A. B., Lacher D. A., Pereira M. A. and Goldblatt P. J. (1986), Mechanisms of chloroform and carbon tetrachloride toxicity in primary cultured mouse hepatocytes. Environ. Health Perspect. **69**, 301–305.

Samanta L., Sahoo A. and Chainy G. B. (1999), Age-related changes in rat testicular oxidative stress parameters by hexachlorocyclohexane. Arch. Toxicol. **73**, 96–107.

Sikkema J., Poolman B., Konings W. N. and de Bont J. A. (1992), Effects of the membrane action of tetralin on the functional and structural properties of artificial and bacterial membranes. J. Bacteriol. **174**, 2986–2992.

Sikkema J., de Bont J. A. and Poolman B. (1995), Mechanism of membrane toxicity of hydrocarbons. Microbiol. Rev. **59**, 201–222.

Søballe B. and Poole R. K. (2000), Ubiquinone limits oxidative stress in *Escherichia coli*. Microbiology **146 (Pt 4)**, 787–796.

Storz G. and Imlay J. A. (1999), Oxidative stress. Curr. Opin. Microbiol. **2**,188–194.

Thurman R. G., Bradford B. U., Iimuro Y., Frankenberg M. V., Knecht K. T., Connor H. D., Adachi Y., Wall C., Arteel G. E., Raleigh J. A., Forman D. T. and Mason R. P. (1999), Mechanisms of alcohol-induced hepatotoxicity: studies in rats. Front. Biosci. **4**, e42–46.

Toraason M., Clark J., Dankovic D., Mathias P., Skaggs S., Walker C. and Werren D. (1999), Oxidative stress

and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene. Toxicology **138**, 43–53.

Tse S. Y., Mak I. T., Weglicki W. B. and Dickens B. F. (1990), Chlorinated aliphatic hydrocarbons promote lipid peroxidation in vascular cells. J. Toxicol. Environ. Health **31**, 217–226.

Ueda I. and Yoshida T. (1999), Hydration of lipid membranes and the action mechanism of anaesthetics and alcohols. Chem. Phys. Lipids **101**, 65–79.

Vercesi A. E., Kowaltowski A. J., Grijalba M. T., Meinicke A. R. and Castilho R. F. (1997), The role of reactive oxygen species in mitochondrial permeability transition. Biosci. Rep. **17**, 43–52.

Verma S. P. and Singhal A. (1991), Low levels of the pesticide, δ-hexachlorocyclohexane, lyses human erythrocytes and alters the organization of membrane lipids and proteins as revealed by Raman spectroscopy. Biochim. Biophys. Acta **1070**, 265–273.

Voie O. A. and Fonnum F. (2000), Effect of polychlorinated biphenyls on production of reactive oxygen species (ROS) in rat synaptosomes. Arch. Toxicol. **73**, 588–593.

Wieland P. and Lauterburg B. H. (1995), Oxidation of mitochondrial proteins and DNA following administration of ethanol. Biochem. Biophys. Res. Commun. **213**, 815–819.

Zhang Q.-M. and Yonei S. (1991), Induction of manganese-superoxide dismutase by membrane-binding drugs in *Escherichia coli*. J. Bacteriol. **173**, 3488–3491.

Zidenberg-Cherr S., Olin K. L., Villanueva J., Tang A., Phinney S. D., Halsted C. H. and Keen C. L. (1991), Ethanol-induced changes in hepatic free radical defense mechanisms and fatty-acid composition in the miniature pig. Hepatology **13**, 1185–1192.