Acrolein and Human Disease: Untangling the Knotty Exposure Scenarios Accompanying Several Diverse Disorders

Philip C Burcham*

Pharmacology, Pharmacy & Anaesthesiology Unit, School of Medicine and Pharmacology, The University of Western Australia, Crawley, WA 6007, Australia.

*Corresponding Author:

Pharmacology, Pharmacy & Anaesthesiology Unit (M510),

School of Medicine and Pharmacology,

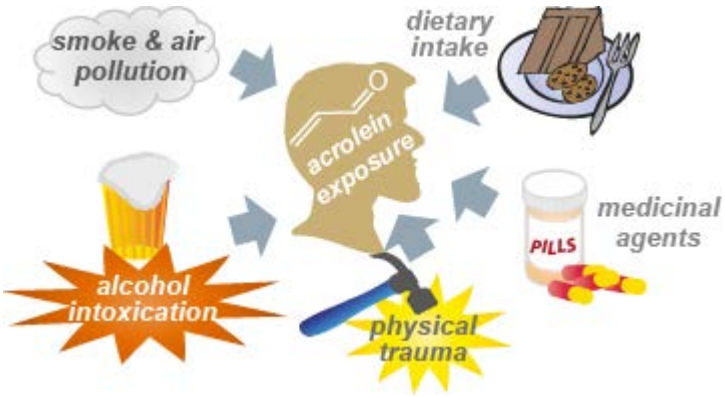
QE2 Medical Centre,

Nedlands, WA 6009, Australia.

Phone: 61-8-9346 2986

Email: philip.burcham@uwa.edu.au

TOC GRAPHIC


smoke & air pollution
dietary intake
acrolein exposure
medicinal agents
PILLS
alcohol intoxication
physical trauma

ABSTRACT

Acrolein is a highly toxic electrophile that participates in many diseases, yet efforts to delineate its precise mechanistic contributions to specific conditions are complicated by its wide distribution within human environments. This Perspective develops the proposal that due to its mixed status as environmental pollutant, metabolic by-product, and endotoxicant which forms via ubiquitous pathophysiological processes, many diseases likely involve acrolein released from multiple sources. Although the category boundaries are indistinct, at least four identifiable exposure scenarios are identifiable. Firstly, in some syndromes, such as those accompanying chronic or acute intoxication with smoke, whatever role acrolein plays in disease pathogenesis mainly traces to exogenous sources such as the combustion of tobacco or other organic matter. A second exposure category involves xenobiotics that undergo metabolism within the body to release acrolein. Still other health conditions, however, involve acrolein that forms via several endogenous pathways, some of which are activated upon intoxication with xenobiotics (i.e. Exposure Category 3) while still others accompany direct physical trauma to body tissues (Exposure Category 4). Further complicating efforts to clarify the role of endogenous acrolein in human disease is the likelihood that many such syndromes are complex phenomena that resemble "chemical mixture exposures" by involving multiple toxic substances simultaneously. This Perspective contends that while recent decades have witnessed much progress in describing the deleterious effects of acrolein at the cellular and molecular levels, more work is needed to define the contributions of different acrolein sources to "real-world" health conditions in human subjects.

CONTENTS

1. INTRODUCTION

Acrolein (prop-2-enal) was identified in 1839 by the Swedish chemist JJ Berzelius during his studies of glycerol decomposition. A pioneer in biological chemistry, Berzelius named the substance for its acrid, choking odour and "oil-like" (*`oleum'*) physicochemical properties.[1] Subsequent structural characterisation revealed that the proximity of the electron-withdrawing carbonyl group to the unsaturated bond confers strong electrophilicity upon acrolein and thus reactivity with nucleophilic molecules.[2] These properties ensure wide use during the synthesis of diverse organic products, but also secure toxicological relevance for acrolein due to its ready reactions with tissue macromolecules. Because it is often encountered as an airborne substance, most interest has focussed upon inhalational exposures, with the lungs, nose and throat bearing the brunt of its irritant effects.[3] The combination of reactivity, volatility and toxicity toward respiratory tissue led to the testing of acrolein as a vesicant-type chemical warfare agent in World War 1.[4]

While interest in its military use subsided early in the 20th Century, recent decades have witnessed growing interest in acrolein toxicology. This resurgence was partly fuelled by the discovery that acrolein contributes to many disorders in ways that transcend established toxicology conventions which typically focus upon human exposures within occupational, environmental or medicinal contexts. In contrast, some significant disorders seem to involve acrolein formation and release from endogenous sources.[5] The possibility that it mediates cell damage in individuals who are not knowingly exposed to exogenous acrolein makes clarifying its role in human disease an intriguing research goal.

This Perspective highlights recent advances by attempting to delineate several exposure paradigms that define the role of acrolein in particular diseases. Our concern is

not primarily to review advances in understanding acrolein toxicity at the molecular level, since this theme is admirably addressed elsewhere.[5] Rather, our goal is to sift through the burgeoning scientific literature on acrolein toxicity with a view to delineating distinct exposure scenarios whereby it contributes to specific disorders. An effort is made to highlight research strategies that were helpful in one context with a view to exploring their potential use in other disease and/or exposure contexts. Before addressing these matters we will briefly summarise the various sources whereby humans might encounter this noxious aldehyde.

2. COMMON SOURCES OF ACROLEIN

The diverse ways in which humans encounter acrolein reflects its wide distribution within human and natural environments. At least four broad routes can be identified.

2.1. Environmental Sources. Acrolein can be present in various environmental settings due to deliberate human activities or its inadvertent release into the atmosphere by human or natural processes. In an example of the former, significant quantities of synthetic acrolein are used as an herbicide to control aquatic weed growth in irrigation canals. Because it decomposes quickly within water, acrolein leaves minimal residues within such settings. In the USA, acrolein is also used as a burrow fumigant to control vertebrate pests such as ground squirrels and prairie dogs.[6] In addition to these agricultural applications, synthetic acrolein finds use as an industrial reagent during the production of acrylate polymers.[1]

Concern over environmental acrolein often centres upon inhalational exposures since it forms during the combustion of most fossil fuels, ensuring a significant role in urban air pollution and associated respiratory problems in dense populations.[7] The combustion of biomass, such as occurs during forest and bush fires, releases large quantities of acrolein as do fires involving synthetic polymers which can release especially high acrolein yields.[8,9] Atmospheric oxidation of the combustion product 1,3-butadiene further contributes to airborne acrolein formation within urban areas.[1]

Inhalation of acrolein-containing indoor air poses additional risks for individuals working in poorly ventilated kitchens where fried food is prepared. Glycerol, a common constituent of cooking oils, was long considered the main precursor within cooktop fumes since it is dehydrated at high temperatures to release acrolein, although recent isotope-labelling studies suggest a likelier origin within the unsaturated backbone of fatty acids.[10] Numerous studies have detected acrolein within domestic and commercial kitchen air samples, with one investigation reporting indoor air concentrations ranging between 26 and 65 micrograms per cubic metre.[11-13] Although such levels fall below occupational threshold limit values (e.g. the TLV for acrolein is 0.1 ppm, or 250 $\mu g/m^3$)[14], recent findings from the Hecht laboratory suggest such kitchen exposures deserve further attention in terms of their possible impact upon human health. These researchers detected elevated levels of acrolein-derived urinary metabolites in non-smoking Asian women who regularly prepare wok-fried foods, suggesting pulmonary acrolein uptake occurs from household sources we encounter in daily life.[15]

Tobacco smoke is the main avoidable source of airborne acrolein because up to 100 µg forms from a single burning cigarette, largely via combustion of sugar-containing leaf

additives.[1,16] Urinary acrolein metabolite levels are typically 5- to 6-fold higher in smokers, suggesting the lungs present minimal anatomical barriers against the absorption of this cell-permeable toxicant.[17] The daily pulmonary barrage with acrolein-containing smoke takes a major toll upon smokers; a recent risk assessment that balanced the concentrations of known tobacco smoke constituents against their toxic potency in lab rodents incriminated acrolein in some 88% of noncancer outcomes (especially respiratory conditions) that accompany tobacco smoking.[18]

Recent research has explored whether e-cigarettes are safer alternatives to conventional cigarettes, as the lack of tobacco combustion likely reduces the risk of first- and second-hand inhalational exposure to noxious smoke constituents.[19] While acrolein-yielding humectants such as glycerol and propylene glycol are often added to the nicotine-containing liquid contained within e-cigarette cartridges, acrolein concentrations within the resulting vapours are typically much lower than corresponding values in cigarette smoke.[20] Recent studies of urinary acrolein metabolite levels in e-cigarette users concur with expectations of lower acrolein exposure compared to conventional smokers.[21,22] Although these differences seem likely to translate into significant health benefits for smokers and bystanders, it has been suggested that a full stocktaking of the harm reduction potential of e-cigarettes could take several decades to complete.[23]

2.2. Metabolic Sources. Two broad classes of compounds undergo metabolic transformations within the body to liberate either acrolein or precursors that yield acrolein upon subsequent spontaneous or enzyme-catalysed reactions. The first group includes various allyl compounds such as the herbicide allyl alcohol and the industrial reagents allyl

chloride and allyl amine. The former is often used to induce metabolite-mediated hepatotoxicity in laboratory animals since it is rapidly oxidised to acrolein by alcohol dehydrogenases within the liver and GI-tract.[24] The prospects for human exposure seem low and published reports of allyl alcohol toxicity are limited to sporadic cases of acute intoxication.[25] On the basis of their urinary metabolite profiles in rodents, allyl bromide, allylamine and allyl cyanide all undergo metabolic conversion to acrolein *in vivo*.[26] The toxicity of allylamine is further addressed in Section 4.2.2 below.

A second category of acrolein-yielding substances are the oxazaphosphorine antineoplastics such as cyclophosphamide and iphosphamide.[27] Following oral administration, cyclophosphamide undergoes hepatic CYP2B1-catalysed conversion to 4-hydroxycyclophosphamide. This hydroxylated metabolite equilibrates with its acyclic tautomer, aldophosphamide, which is either further metabolised to inactive products or undergoes rearrangement to release phosphoramide mustard and acrolein. The former likely contributes to antitumor outcomes while the latter is implicated in dose-limiting bladder damage that afflicts cyclophosphamide recipients (*see below*).[28]

2.3. Dietary Sources. Humans daily ingest small quantities of acrolein due to its presence in many foods including cooked poultry, molasses, salted pork, fish, roasted coffee, potato chips and berries.[1] Acrolein is also present within most alcoholic beverages including beer, whisky, brandy and wine.[29] High acrolein levels in red wine contribute to the astringent "pepper" flavour that emerges during prolonged storage in the presence of anaerobic microorganisms.[30] Resembling its role as an acrolein precursor in other contexts (e.g. tobacco leaves), glycerol it is again a likely culprit in oenological contexts: present at

concentrations ranging from 5-8 g/L, it undergoes a 2-step transformation to form acrolein within aged red wines.[30]

As highlighted during discussion of kitchen air quality in *Section 2.1*, acrolein forms upon heating unsaturated food oils at high temperatures, and these processes also ensure its presence in many fried foodstuffs. Baked sugary foods also contain acrolein due to the high-temperature degradation of reactive precursors such as hydroxy-acetone.[1] Despite these considerations, acrolein levels within most common foodstuffs are usually quite low, and since the liver and gastrointestinal tract wall express many detoxication enzymes that mitigate the uptake of ingested xenobiotics, the extent to which dietary sources contribute to circulating free acrolein levels is uncertain. An early study of the fate of radiolabelled acrolein [2,3-C^{14}] when administered by either i.v. injection or oral gavage to fasted Sprague-Dawley rats revealed that lower tissue accumulation occurred via the oral route, suggesting the GI-tract helps to minimise the oral absorption of ingested acrolein.[31] In one of the few modern studies of the effect of dietary practices upon urinary metabolite profiles, German researchers reported a clear increase in acrolein-derived mercapturates in human urine following the consumption of potato crisps.[32] Whether the glutathione conjugates from which these urinary metabolites derive within the gut wall and liver or instead reflect the subsequent conjugative metabolism of ingested free acrolein within peripheral tissues following its distribution via the circulation is unknown.

2.4. Endogenous Sources. The recognition that acrolein forms via several endogenous processes, some of which are amplified during cellular stress, has helped fuel growing interest in acrolein toxicity. These include the oxidation-prone sulfur amino acid

methionine, which undergoes classic Strecker degradation chemistry to ultimately form a methional sulfoxide intermediate that can decompose to liberate acrolein.[1] Another route accompanying inflammatory states involving neutrophil recruitment features the myeloperoxidase-catalysed oxidation of the amino acid threonine.[33] In addition, acrolein forms during the oxidation of spermine and spermidine, members of a ubiquitous family of polyamines that exert broad regulatory actions on many cell functions.[34] Acrolein formation via polyamine oxidation is implicated in such degenerative conditions as brain infarction and renal failure.[35,36]

The main endogenous acrolein-yielding process in terms of its potential involvement in many pathophysiological processes is likely lipid peroxidation (LPO), an autocatalytic degradative process to which unsaturated lipids within cell membranes and fat storage droplets are prone.[37] LPO typically accompanies the induction of oxidative stress and is thus implicated in many health conditions, especially those involving chronic inflammation.[38] Acrolein likely forms from autooxidised lipids along the mechanistic lines suggested by Esterbauer involving β-cleavage of alkoxyl radical intermediates.[1,39] (**Scheme 1**). As a non-orchestrated process, LPO generates many products including numerous electrophiles which share the ability of acrolein to attack cell macromolecules to form adducts. We will briefly explore the toxicological implications of the cascading nature of LPO below.

Most data which incriminates endogenous acrolein in human disease derives from the chemical quantitation of acrolein-derived protein or DNA adducts within target tissues. Such damage likely accompanies routine aerobic metabolism, the ubiquity of which likely explains the “baseline” damage detected in control subjects who are not knowingly

exposed to exogenous acrolein (see below). To date, the question of which specific endogenous pathways contribute to baseline levels of acrolein-derived protein or DNA adducts has received minimal attention. Similarly, the origins of urinary acrolein metabolites in nonsmokers are poorly understood since little is known concerning the extent to which endogenously-produced acrolein escapes its site of origin (e.g. an inflamed joint) to undergo conjugative metabolism in peripheral tissues before excretion by the kidneys. Such knowledge is pertinent to the question of which biomarkers are most appropriate during studies of acrolein participation in particular disease settings.

3. TRACKING ACROLEIN WITHIN THE BODY – FREE LEVELS, METABOLITES AND ADDUCTS

Chemical toxicity is often conceptualised in terms of the "toxicological paradigm" that posits a succession of stages in the expression of chemically-induced disease (**Scheme 2**). Underlying such frameworks is an expectation that analytical determination of specific markers at each step strengthens the association of specific chemicals with the pathogenesis of a particular clinical syndrome. Progression in understanding the toxicology of any given substance thus involves identifying new biomarkers or diagnostic indicators at each stage of the paradigm. This general approach has proven very useful during studies of acrolein toxicology, and it is helpful to briefly review progress in defining markers at each stage of its toxicological continuum. This Perspective focuses especially upon progress at the toxicokinetic end of the spectrum (**Scheme 2**), namely the development of tools to define the "Internal Dose" and "Biologically Effective Dose" of acrolein.

3.1. *Acrolein Exposure Markers.* Assessment of acrolein exposure usually entails measurement of its concentrations within ambient air, ingested food, water or beverages. Due to its high vapour pressure and efficient pulmonary uptake, acrolein concentrations in air help predict human exposure via the inhalational route, although outdoor air concentrations are somewhat dynamic since acrolein is degraded through photochemical reactions with various atmospheric radicals.[4] Similarly, the "aging" of tobacco smoke within indoor settings ensures toxicant concentrations can change with time via reactions with gas radicals, although recent studies suggest many volatile organic compounds such as acrolein are relatively stable in such environments.[40]

Knowledge of acrolein concentrations within foodstuffs can assist estimates of dietary exposure via the oral route. A Tolerable Daily Intake (TDI) of 7.5 μg/kg body weight was derived from No Observed Adverse Effect Level (NOAEL) estimates that were obtained using chronic rodent data.[41] The extent to which the food matrix influences the oral bioavailability of acrolein has received minimal attention, and its reactivity with nucleophilic food components may complicate estimation of human exposures based on "free" acrolein concentrations in food.[42]

3.2. *Markers of the "Internal Dose."* The internal dose of a compound is typically estimated by quantitating the parent compound and its metabolites within biofluids collected from exposed individuals. In the case of acrolein, such efforts are complicated by the strong electrophilicity that facilitates reactions with glutathione or tissue macromolecules. The readiness with which blood proteins sustain acrolein adduction[43,44]

likely lowers free concentrations within the circulation, which may explain why plasma levels of free acrolein are not widely reported within the scientific literature. Yet measurements of free acrolein are useful in certain physiological contexts, most notably within the urine of patients receiving acrolein-yielding oxazaphosphorine drugs.[45,46] The bladder environment is somewhat distinctive because acrolein can form directly within renal filtrate via the decomposition of unstable cyclophosphamide metabolites, plus the low protein concentrations in urine minimise acrolein-trapping reactions with protein nucleophiles.

Compared to the limitations attending estimation of its "free" levels in blood and tissues, measurements of acrolein metabolites are useful indicators of the "internal dose" in many contexts (**Scheme 2**). The main metabolic fate of acrolein involves reaction of the 2,3-double bond with the cysteinyl thiol of glutathione via nonenzymatic or glutathione-*S*-transferase catalysed reactions.[47] In the typical manner of xenobiotic-derived glutathione conjugates, the primary acrolein-glutathione metabolite undergoes proteolytic cleavage and *N*-acetylation within kidney to form the mercapturate *S*-(3-oxopropyl)-*N*-acetyl cysteine (or S-(3- oxopropyl)mercapturic acid, OPMA). Competing reactions for the aldehydic group of OPMA generate two additional mercapturates; a reductive pathway to form *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine (or 3-hydroxypropylmercapturic acid, 3HPMA), the major urinary metabolite of acrolein, and an oxidative route which yields *N*-acetyl-*S*-[2-carboxyethyl]-L-cysteine (or 2-carboxyethylmercapturic acid, CEMA), a minor urinary metabolite **(Scheme 3)**. 3HPMA and CEMA hold considerable promise as urinary biomarkers of "whole body" acrolein exposure in diverse disease settings, with the caveat that existing methods for their determination do not reveal whether any acrolein that is

excreted in a given disease state is of endogenous origin, exogenous origin, or a combination of both.

3.3. *Markers of the "Biologically Effective Dose."* For xenobiotics that are metabolised to reactive intermediates, the "biologically effective dose" represents the proportion of the administered dose that having undergone bioactivation, escapes detoxication and forms covalent adducts within cellular targets. On classic understandings of chemical toxicity according to the "covalent binding hypothesis," knowing the concentrations of macromolecular adducts within different tissues can reveal the vulnerability of that organ to toxic outcomes.[48] In early studies of the fate of radiolabelled acrolein in rats, the proportion of acrolein that was stably incorporated into tissues scarcely exceeded 1% of the administered dose,[31] a figure that concurs with low levels of incorporation of bioactivation-dependent toxicants into target proteins determined using modern mass spectrometric techniques.[49] Because this low proportion of the administered dose is presumed to drive the pathological responses to xenobiotics, modern toxicology devotes considerable effort to developing analytical methods that permit quantification of compound-derived adducts within exposed cells and tissues.[50] In the case of acrolein, this task is complicated by the strong electrophilicity which ensures adduction occurs at many nucleophilic residues within cell macromolecules. A range of adducts formed during alkylation of protein nucleophiles is shown in **Scheme 4**. Although it is presently beyond our capabilities to quantify all of these species simultaneously within tissues, the development of assays to allow measurement of the primary species has assisted study of acrolein involvement in many diseases.

In addition to quantifying the "biologically effective dose" by means of adduct measurements, identifying the specific proteins that sustain adduction adds extra layers of mechanistic insight since it can reveal the cellular networks that are disrupted during acrolein toxicity. Because acrolein behaves as a strong "soft" electrophile, reactions with soft nucleophiles including cysteine thiol groups in cell proteins are favoured targets.[51] As cytosol is a relatively reduced environment in which cysteine thiols predominate relative to disulphide bridges, reactive metabolites with soft electrophilic character tend to favour reactions with cysteine-containing cytosolic proteins. Indeed, it is possible thiol-containing cytosolic proteins serve as "sponges" for electrophilic intermediates, thereby protecting harder nucleophilic centres within critical cell macromolecules in essential organelles.[52] On this understanding, identifying the subset of critical targets and not merely the most abundant cytosolic targets provides the greatest mechanistic insight into electrophile-mediated toxic syndromes. The significance of such considerations will become apparent during our discussion of alcoholic liver disease below.

3.4. *Markers of "Early Biological Effect."* The chemical damage inflicted upon cellular constituents by electrophilic intermediates elicits a complex series of deleterious changes that can be studied at multiple levels of biological organisation ranging from the molecular (e.g. adduction of a protein target), biochemical (e.g. activation of caspase activity), or histological (e.g. changes in immune cell numbers, tissue ultrastructure, etc). During modern studies, such classic methods are combined with systems toxicology approaches that incorporate analysis of global metabolomic, lipidomic or other 'omic datasets to construct sophisticated models of chemically induced disease.[53] While many insights into

acrolein toxicity have emerged from such studies, the indiscriminate reactivity of acrolein and its ability to damage many cellular targets ensures that a unifying theory which encompasses its diverse biological effects under a single disease mechanism is difficult to formulate.[5] These realities can complicate efforts to define its role in some common diseases, a topic to which we now turn.

4. ACROLEIN AND DISEASE: FOUR EXPOSURE SCENARIOS

The remainder of this Perspective explores four broad exposure scenarios that have emerged during studies of the role of acrolein in particular diseases (**Table 1**). Rather than attempting to address all possible conditions in which acrolein may participate, attention is given to select disorders that in the author's opinion best exemplify a particular exposure paradigm. Within each category, discussion focuses on recent studies that have advanced knowledge of acrolein involvement in a designated disease by using definitive methods to confirm a role for acrolein at the level of either the "Internal Dose" or the "Biologically Effective Dose." While four exposure classes are highlighted for the sake of clarity, they are unlikely to be water-tight and overlap may exist between them.

4.1. Toxic syndromes involving acrolein of primarily exogenous origin

Health conditions within this category involve acrolein that enters the body from an external source. Several such routes including dietary and environmental sources were summarised in Section 2 above. With the exception of relatively small numbers of workers in specific industries who risk encountering synthetic acrolein via their daily work (e.g.

industrial plant workers, aquatic herbicide or vertebrate pesticide applicators, etc), the over-riding source of exogenous acrolein for most individuals is inhaled smoke. Two major scenarios can be distinguished which differ significantly in terms of the duration and magnitude of smoke exposure, namely slowly progressing conditions that reflect chronic exposure to tobacco smoke and life threatening disorders that follow acute intoxication with environmental smoke. It is important to note that while acrolein is likely a key driver of some significant toxic responses to acutely or chronically inhaled smoke, neither of these scenarios remotely resemble "acrolein only" exposures. Because the combustion of tobacco and other biological matter generates a plethora of toxicants, any manifestation of toxicity accompanying smoke exposure surely involves a complex interplay between multiple noxious substances. Nevertheless, the high abundance of acrolein within many forms of smoke together with its pronounced chemical reactivity and corrosive properties towards respiratory tissue ensures a substantive contribution to some significant smoke-related pulmonary syndromes.

4.1.1. *Chronic Lung Injury by Tobacco Smoke.*

The emergence of physiologically-based pharmacokinetic models that use imaging of the human respiratory tract to construct computational simulations of tobacco smoking have reinforced a key role for acrolein in smoking-related lung injury.[54] By modelling airflow and the distribution of catabolic enzymes, researchers could estimate aldehyde concentrations at different anatomical sites throughout the human airways, with the results suggesting that acrolein can penetrate deep into the bronchiolar zones following inhalation via the nasal or oral routes.[54] These findings concur with longstanding rodent studies that

described irritation of nasal tissue and bronchiolar airways following inhalational exposure to acrolein or tobacco smoke.[55]

The main pathological outcome in which acrolein is implicated in smokers is chronic obstructive pulmonary disorder (COPD), a devastating condition that involves a progressive loss of core respiratory functions. Despite increased research and clinical attention, COPD remains among the top five global causes of morbidity and mortality.[56] While COPD occurs most frequently among smokers, chronic exposure to other forms of air pollution including fumes emitted from poor quality stoves can induce a similar syndrome in low socioeconomic settings.[57] A key diagnostic feature of COPD is a decline in the FEV_1, the volume of air that can be forcibly expired in a 1-second interval. The perceived inability to satisfy respiratory needs in COPD patients confers a distressing sensation of ‘‘breathlessness’’ that reflects permanent enlargement of distal respiratory air spaces due to progressive emphysematous destruction of the alveolar wall.[58] Other COPD characteristics include mucus hypersecretion, small airways obstruction, recurrent coughing, vulnerability to respiratory infections and pulmonary hypertension. The condition is progressive in nature but involves periodic exacerbations that promote worsening lung function, exercise intolerance, frequent hospital admissions and increased mortality. COPD takes a devastating toll upon individuals, with the economic impact alone due to early retirement estimated at an average loss of $316,000 in lifetime earnings.[59] The societal impact including the burden upon hospitals is also substantive, especially during the final months of COPD.[60] The pharmacotherapy of COPD is challenging since unlike the drug-responsive airflow restrictions that accompany asthma, bronchodilators afford only modest benefits.[61]

The lung pathology underlying COPD is an exaggeration of the low-grade inflammatory cell infiltration to the bronchi and peripheral lung that occurs in most smokers. For reasons that remain unclear, for some vulnerable smokers this process is amplified and accompanied by a tissue-remodelling process that elicits a cluster of COPD symptoms. The evolution to an 'abnormal' inflammatory response is likely driven by disparities in the protease–antiprotease and oxidant–antioxidant balance as well as epigenetic changes in gene expression that regulate the influx of neutrophils, macrophages, and lymphocytes.[62] This environment triggers pro-inflammatory cytokine production and apoptosis within lung parenchyma, eliciting alveolar destruction and remodelling of the small airways.

The evidence suggesting acrolein involvement in COPD is broad and multifaceted.[63] Firstly, among the many constituents of tobacco smoke, acrolein is one of the most potent stimulants of mucus hypersecretion, a major feature of COPD.[64] Produced by goblet cells within secretory ducts in the large airways, mucus is rich in highly glycosylated mucin proteins that protect the epithelium against respiratory irritants, although its production rises to uncontrolled levels in COPD and other lung disorders. MUC5AC, the key mucin-producing gene in mammalian lung, is strongly upregulated in murine lung epithelium upon chronic exposure to 2 ppm airborne acrolein.[65] Low acrolein concentrations also induce MUC5AC mRNA levels in human lung epithelial cells.[66] MUC5AC upregulation by acrolein likely proceeds by activation of the mitogen-activated protein kinase (MAPK) pathway by epidermal growth factor receptor (EGFR) ligands formed via the degradation of the extracellular matrix by various metalloproteinases.[67,68]

In vitro studies directly implicate tobacco smoke acrolein in regulating the production of key pro-inflammatory mediators such as the neutrophil attractant IL-8 by lung epithelial cells.[3] At low acrolein concentrations, an upregulation of IL-8 production due to MAPK activation predominates while at higher levels of exposure, adduction of proteins that regulate NF-kB transcriptional activity strongly suppresses IL-8 release. At any given level of tobacco smoke exposure, overall IL-8 production may reflect the nett effect of acrolein on these opposing pathways (**Scheme 5**).[3]

Acrolein also likely participates in the macrophage adhesion and activation that defines the alveolar destruction phase of COPD.[69] Early microarray analysis of COPD lung samples identified the pro-inflammatory transcription factor Egr1 as a strongly predictive COPD marker.[70] A likely role for acrolein in Egr1 upregulation was suggested by microarray studies in A549 lung cells.[71] Acrolein may thus foster COPD pathogenesis by driving the expression of this pro-inflammatory transcription factor, the activation of which in cells exposed to cigarette smoke extracts leads to inflammatory cytokine release and metalloproteinase activation.[72,73]

Associations between acrolein and COPD are strengthened by data from animal models or human tissues that help to define the "biologically effective dose" in terms of DNA or protein adduct levels. In a notable rodent study, Conklin and associates used immunochemical approaches to detect acrolein-adducted proteins within the lungs of mice that were subjected to a 5 h inhalational exposure to tobacco smoke.[74] Intriguingly, acrolein-adducted proteins were prevalent within the lungs of air-exposed controls, suggesting the involvement of endogenously-produced acrolein in "baseline" modifications to the lung proteome. Significant smoke-related increases in acrolein adduction occurred in

several lung proteins with apparent masses of 22, 30, 75, and 250 kDa.[74] Intriguingly, less adduct immunostaining was noted in mice that were sacrificed 24 h after the final exposure to tobacco smoke compared to tissues taken immediately after a 5-h exposure, suggesting the acrolein adducts were unstable within the lung tissue.[74] While the authors attributed this effect to the rapid removal or repair of adducted proteins, the chemical decomposition or consumption of adducts via crosslinking reactions represent further possibilities.[75] Comparable patterns of protein adduction were noted in mice following a 5 hr inhalational exposure to pure acrolein (5 ppm) in the same study. This work by Conklin and associates confirms the formation of acrolein adducts in lung tissue during tobacco smoke exposure and highlights the need for ongoing work to define their pathological contributions and biological fate using quantitative analytical approaches.

Although their disease contributions differ from those of protein adducts, knowledge of DNA adduct concentrations also provide insight into the biologically effective dose of acrolein in the lungs of smokers. In one study of this kind, Hecht and associates used liquid chromatography-electrospray ionization-tandem mass spectrometry to quantify acrolein-derived DNA adducts in lung biopsies collected during surgical procedures performed on smokers or ex-smokers.[76] Although the sample numbers were small, the study findings did not suggest a significant difference in adduct levels between the two groups, a finding that has relevance to controversies concerning the status of acrolein as a human lung carcinogen in tobacco smokers.[77,78] Such technologies hold promise for future use to define acrolein adduct levels in COPD samples, explore their intra-tissue distribution within human airways or the dose-dependence of DNA damage relative to smoking behaviours.

While COPD seems a useful disease in which to clarify the contribution of exogenous acrolein to human disease, several considerations complicate these efforts. First, while the rich chemical complexity of tobacco combustion ensures multiple chemicals likely contribute to COPD pathogenesis, due to the limitations in current technologies, many analytical studies performed to date have focussed upon macromolecular damage by individual chemicals. Future advancement in defining the pathogenic roles of noxious smoke toxicants in COPD await the development of adductome-characterising tools that provide a fuller inventory of macromolecular damage. Biotin hydrazide protein capture approaches that enabled study of the role of multiple carbonylating electrophiles in alcoholic liver disease will be briefly reviewed in Section 4.3.1, and seemingly hold much promise for studies of lung damage during tobacco smoking.

Second, although smokers expose themselves to exogenous smoke-borne acrolein in the first instance, the inherent chemical properties of tobacco smoke and tissue responses to smoke toxicants ensure that COPD is complicated by oxidative stress that likely promotes endogenous acrolein formation via LPO. The tar component of tobacco smoke contains pro-oxidant poly-quinoid complexes which subject the smoker's lung to persistent superoxide production via redox-cycling chemistry.[79] In addition, the activation of neutrophils and macrophages that produce prodigious quantities of superoxide radicals further subjects the smoker's lung to oxidative stress. A prevailing pro-oxidative environment within the COPD lung is supported by measurements of LPO products such as 8-isoprostanes within sputum.[80] Although acrolein production via LPO seems likely under these conditions, few if any studies have definitively tracked contributions by endogenous versus exogenous acrolein in COPD. This consideration also applies to some other disease

syndromes considered below, including those accompanying acute intoxication with high doses of smoke.

4.1.2. *Acute Lung Injury by Environmental Smoke*.

Exogenous acrolein exposure likely participates strongly in some cases of Smoke Inhalation Injury (SII), a life-threatening condition seen in victims of domestic and environmental fires. In contrast to the slowly developing pulmonary dysfunction that typifies COPD, fire victims who acutely inhale high doses of smoke are vulnerable to a rapidly progressing pneumotoxic syndrome that is immediately life threatening, with death frequently occurring within 3 to 5 days of smoke intoxication due to multi-organ failure and other complications. SII is typically a major contributor to mortality during large fire-related disasters.[81] Across a range of patient ages, SII victims exhibit much higher mortalities relative to those with burns of comparable severity in the absence of smoke inhalation.[82]

SII is a variable clinical syndrome due to the unpredictability of various determinants of disease severity. These can include the amount of soot deposited within the airways; the presence of thermal burns; the degree of tissue asphyxiation caused by inhaled respiratory inhibitors such as carbon monoxide; the quantity of inhaled smoke, its chemical profile and the nature of the combustible material.[83] As the port of entry, the lung is very vulnerable to pulmonary oedema upon acute smoke intoxication. Respiratory failure can involve a latency of 24–72 h and involves the emergence of severe bronchorrhea, bronchospasm, breathing abnormalities, and retrograde alveolar flooding.[84,85] Pulmonary oedema in SII

victims resembles other forms of acute lung injury and involves a loss of vascular and epithelial permeability and impairment of ion and fluid transporters that normally maintain ‘‘dry’’ alveolar airspaces.[86] Production of proinflammatory cytokines also exacerbates the acute phase of SII.[87,88]

The data implicating acrolein in SII-related pulmonary oedema is long-standing.[3] In early canine studies, Zikria and associates noted that the oedematogenic potency of smoke produced from different combustible materials correlated closely with its acrolein content.[89] In later work conducted in anesthetized sheep, Hales and associates used synthetic smoke containing different combustion by-products to incriminate acrolein as a key oedematogenic constituent.[90-92] This large animal model also allowed identification of leukotrienes as mediators of oedematogenesis by acrolein-containing synthetic smoke.[93]

More recent work has explored the molecular basis whereby acrolein might promote the onset of pulmonary oedema. In studies conducted in A549 cells, a lung-derived tumour cell line of alveolar epithelial origin, intermediate filaments and vimentin were highly vulnerable to adduction by acrolein, with such damage correlating with a functional loss of cellular adhesive properties, a phenomenon that if it has an *in vivo* correlate, could render respiratory epithelium less watertight.[94] In a comprehensive *in vivo* study, Leikauf and associates explored the molecular factors underlying the vulnerability of mice to inhaled acrolein, and identified claudin-5 as a key determinant of acrolein sensitivity.[95] An integral membrane protein, claudin-5 helps maintain endothelial integrity within pulmonary vasculature, hence disruption of this pathway may confer the eroded vascular integrity that accompanies pulmonary oedema during SII.

Despite experimental support suggesting a role for smoke-borne acrolein in SII, some key issues require attention before the association is fully established. Presently, few if any diagnostic methods allow confirmation of acrolein involvement in any particular SII patient. Much of the unpredictability accompanying the clinical evaluation of SII reflects the unknown chemical composition of the smoke to which an individual fire victim is exposed, hence the availability of methods for the rapid estimation of the "internal dose" of acrolein could assist the triage of patients within emergency departments. Future studies might thus explore whether acrolein-mercapturate concentrations within urine samples could provide useful diagnostic insight of this kind. Similarly, better definition of the "biologically effective dose" in relation to oedema outcomes would also be valuable; to date few studies have obtained quantitative descriptions of the levels or distribution of acrolein-derived macromolecular adducts within the lungs of acutely smoke-intoxicated rodents or human subjects. Such knowledge would help us better judge the status of smoke-borne acrolein as a mediator of this devastating condition.

4.2. Acrolein exposure via xenobiotic biotransformation

A second scenario under which acrolein contributes to pathological outcomes occurs in individuals exposed to certain xenobiotics that generate the noxious electrophile during their metabolism within the body. As highlighted in Section 2.2, acrolein-yielding xenobiotics such as allyl compounds and oxazaphosphorine antineoplastic drugs have long been known to pose high risks to humans. Although exposure to the former compound class is likely restricted to relatively few workers in specific industries, cyclophosphamide remains widely used during the treatment of solid and haematological tumours, hence the

release of acrolein precursors following the CYP2B6-mediated metabolic clearance of cyclophosphamide ensures a substantial role in the grave side-effects that plague this patient group.

4.2.1. Bladder cystitis and cyclophosphamide

The role of acrolein in the dose-limiting haemorrhagic cystitis that accompanies cyclophosphamide treatment was inferred using animal studies several decades ago.[96] As noted in Section 3.2, determination of acrolein concentrations within urine samples from cyclophosphamide-treated patients helped delineate the "internal dose" of acrolein that mediates this toxico-clinical syndrome in humans.[45,46] To date however, relatively few studies have sought to define the "biologically effective dose" for cyclophosphamide-derived acrolein by quantifying acrolein adducts within toxicity prone tissues following cyclophosphamide administration. In one key study, Conklin and associates used an immunochemical approach to analyse the intra-tissue distribution of acrolein-adducted proteins within cross-sections prepared from the urinary bladders of mice 24 h after cyclophosphamide administration (100 to 300 mg/kg, i.p.).[97] Consistent with the onset of cystitis, histological evaluation revealed substantial urothelial exfoliation and extensive haemorrhaging of the lamina propria.[97] An increase in the wet-weight of excised bladders confirmed the onset of tissue oedema. Confirming prior studies that implicated acrolein in cyclophosphamide urotoxicity, protein adducts co-localised with sites of vascular damage and degradation of connective tissue.[97] Analysis of tissue homogenates via Western blotting revealed that acrolein adduction of several proteins increased in mouse bladder 4 h after cyclophosphamide administration. Tissue damage and protein adduction were further

increased in drug-treated mice that lacked the gene for glutathione-S-transferase P, a GST isoform that that displays high conjugative activity towards acrolein.[97]

As noted in Section 4.1, endogenously-produced acrolein complicates evaluation of the role of exogenous acrolein in smoke-associated pathologies, and similar considerations apply when judging its contribution to cyclophosphamide urotoxicity. Conklin and associates noted significant acrolein adduction of bladder tissue proteins in control animals, with the levels further increased in GSTP-deficient animals.[97] Others reported an increase in malondialdehyde concentrations within the urinary bladders of cyclophosphamide-treated rats under conditions which increased levels of acrolein-adducted proteins, indicating that LPO does occur in this tissue context.[98] While such findings raise the possibility that LPO-derived acrolein contributes to protein adduction during cyclophosphamide urotoxicity, it seems likely cyclophosphamide-derived acrolein is the over-riding mediator of bladder damage in this syndrome, and that any acrolein that forms via tissue injury is of secondary significance.

Although most attention has focussed on its role in bladder cystitis, researchers have also explored whether acrolein mediates various systemic toxicities that plague recipients of cyclophosphamide-containing chemotherapy regimens. Cardiotoxicity, for example, accompanies the use of many chemotherapy drugs including cyclophosphamide.[99] In rodents, high dose cyclophosphamide induces a hyperlipidemic cardiomyopathic response that reproduces features of the human syndrome.[100] Consistent with a contribution to cardiotoxicity, acrolein-adducted proteins have been detected within plasma and the aorta wall of cyclophosphamide-treated rats.[101] In more recent work, Conklin and associates used antibody- and mass spectrometric-based approaches to

characterise acrolein-adducted proteins within cardiac lysates prepared from cyclophosphamide-treated rats.[102] Immunoprecipitation of the tissue extracts allowed identification of some 20 acrolein-adducted proteins, a conspicuous member of which was the heme-containing oxygen-carrier myoglobin.[102] Myoglobin modification is implicated in the pro-atherogenic effects of other α,β-unsaturated aldehydes because it likely promotes peroxidative damage to lipoproteins.[103] In the Conklin study, acrolein modification of myocardial proteins correlated with cyclophosphamide-associated elevations in plasma creatine kinase, a classic marker of chemically-induced heart damage, as well as scores assigned during the evaluation of heart muscle slices for apoptotic cells using the TUNEL assay.[102] Echocardiograms collected during ultrasonography of rats further revealed cyclophosphamide-associated deterioration in cardiac function.[102] Exacerbation of the cyclophosphamide-induced cardiac changes in GSTP-deficient mice reinforced a significant role for acrolein.[102]

Taken together, these studies strengthen direct participation by acrolein in various toxicities that accompany dosing with cyclophosphamide, a widely used cytotoxic antineoplastic agent. While more work is needed using definitive methodologies to bring quantitative rigour to the association of acrolein with these toxicities - and to tease apart the quantitative contributions of endogenous versus exogenous acrolein sources - the recent findings reinforce and enlarge classic understandings of acrolein involvement in the serious side-effects of this widely used chemotherapy agent.

4.2.2. *Atherosclerosis and allylamine*.

Allylamine is used as a synthetic reagent during the production of various materials including ion-exchange resins and pharmaceutical agents. Although human exposures

seem mostly limited to occupational settings, isolated reports suggest allylamine is present in animal-derived foodstuffs.[104] In exposed rodents, allylamine undergoes rapid semicarbazide-sensitive amine oxidase-catalysed conversion to acrolein, with 3-HPMA identified as the main urinary metabolite.[105] Across multiple species of laboratory animals, allylamine causes extensive damage to heart tissue and vascular beds, ensuring wide use as an experimental research tool because of the similarity between allylamine-induced lesions and those accompanying human myocardial necrosis, atherosclerosis and acute vasculitis.[106] The association of atherosclerotic lesions with allylamine exposure is interesting since acrolein can induce vasospasms in human blood vessels, a type of spontaneous vasoconstriction that accompanies atherosclerosis.[107] As indicated in the preceding section, atherosclerosis also accompanies human exposure to the acrolein-forming cytotoxic agent cyclophosphamide.[99] Although the vascular protein targets for acrolein adduction have not been characterised in animal models of allylamine-induced atherosclerosis, several classic studies have detected acrolein adducts within human atherosclerotic lesions.[37,108] The high levels of acrolein adducts within macrophage-derived foam cells and the abnormal neointima of arterial vessels led Uchida and associates to implicate LPO as the likely source of the proatherogenic acrolein.[37, 108] Formation via myeloperoxidase-coupled L-threonine oxidation is another likely source within atherosclerotic lesions.[33] The formation of lipid-laden cells is thought to reflect impaired reverse transport of cholesterol from vascular wall macrophages to the liver following acrolein adduction of critical lysine groups in apoA-1 within atherosclerotic lesions.[109] Taken together, these findings suggest the proatherogenic properties of acrolein likely involve a combination of factors including impaired cholesterol efflux, disrupted

vasoreactivity and vasospasm.

4.3. Endogenous acrolein exposure as a pathophysiological response to xenobiotic intoxication

This category of exposures superficially resembles the preceding class since it is initiated by xenobiotics that promote acrolein formation within body tissues. However, in the former category the xenobiotic itself acts as acrolein donor since the noxious aldehyde is liberated from within its molecular structure during biotransformation. The present category instead proceeds via xenobiotic-induced damage to cell structures, with the acrolein originating as a fragment of a cell macromolecule which undergoes chemical degradation during the toxic syndrome. As highlighted in Section 2.4, the most prevalent endogenous acrolein-forming process is likely LPO (**Scheme 1**), the ubiquity of which within tissues that are experiencing oxidative stress ensures it contributes to many diseases. Although numerous conditions could be highlighted, we will focus upon a toxic syndrome of broad societal relevance, alcoholic liver disease.

4.3.1. Alcoholic liver disease (ALD)

Liver disease is a key contributor to mortality in alcoholics, accounting for some 55% of alcohol-related fatalities.[110] Death is the culmination of successive pathological changes within the livers of long-term heavy drinkers, some of which may reverse upon stopping alcohol consumption, while others are largely irreversible (**Scheme 6**).[111] The first identifiable change seen within a week or two of heavy drinking (>60 g/day) is *fatty liver*

or large-droplet (macrovesicular) hepatic steatosis which initially appears within centrilobular (Zone 3) hepatocytes and eventually extends throughout the liver. Due to the hepatomegaly resulting from the lipid accumulation, affected individuals may experience mild abdominal discomfort and tight waistlines. While reproducing the full spectrum of ALD in rodents is challenging, the steatotic phase is achieved in rats using gastric cannulas to deliver high calorie ethanol-containing liquid diets.[112] Although fatty liver was once considered a benign hepatic abnormality since it is mostly reversible upon cessation of alcohol consumption, some 20 to 40% of affected patients who continue heavy drinking progress to a clinically-significant fibrotic syndrome involving tissue inflammation, fibrosis and liver cell necrosis. (**Scheme 6**).[113] Patients often present with mild, asymptomatic disease but with time can experience increasingly debilitating symptoms including hyperbilirubinemia, fatigue, malaise, fever and various intestinal complaints that promote malnutrition including nausea, anorexia, vomiting, and pain. The potentially life-threatening pre-cirrhotic liver injury seen in heavy drinkers is often termed *alcoholic hepatitis*.[114] Unfortunate individuals eventually progress to the end-stage of ALD, cirrhosis, which features massive irreversible scarring due to the progressive replacement of dead hepatocytes with scar tissue.[110] The onset of cell death during ALD proceeds via multiple mechanisms including a strong immunopathological component involving gut endotoxins and subsequent Toll receptor-mediated Kupffer cell activation and the release of pro-inflammatory cytokines.[115] Alcoholic cirrhosis also features recurring cycles of proliferative activity that form nodules of regenerating cells in a futile attempt to restore normal tissue architecture. For the small proportion of ALD patients who survive the cirrhosis phase, many finally succumb to hepatic carcinoma (**Scheme 6**).

The many ways whereby free radicals form within the livers of heavy drinkers ensure oxidative stress features prominently in ALD. Major pathways include shifts in the redox state of pyrimidine nucleotides that facilitate the production of reactive oxygen by the mitochondrial respiratory chain[116]; free radical release during ethanol metabolism by the inducible CYP2E1 system[117]; and production of superoxide radicals by NADPH oxidase in activated phagocytes.[118] This persistent pro-oxidative environment ensures a prolonged susceptibility to LPO during ALD, with hepatocytes subjected to an ongoing barrage of reactive α,β–unsaturated aldehydes such as acrolein. The hepatic proteome of heavy drinkers thus contains a rich array of post-translational modifications, including adducts formed by multiple carbonylating aldehydes as well as oxidised fatty acids.[119]

Recent improvements in mass spectrometry and new chemical tools for the recovery of adducted proteins have allowed global study of protein damage in ALD that covers a large proportion of the hepatic proteome. Because acrolein and other α,β–unsaturated aldehydes react with protein nucleophiles to form Michael adducts, carbonyl-trapping hydrazide-based enrichment strategies permit the recovery and identification of even low abundance damaged proteins at different stages of ALD. Although most attention has focussed upon such carbonylating LPO products as malondialdehyde and 4-hydroxynonenal, use of biotin hydrazide-based analysis by Petersen and associates recently allowed the detection of acrolein-adducted proteins within a murine ALD model.[120]

Using a liquid–based diet that reproduced human patterns of extended binge drinking, mice were subjected to a 6-week regimen during which the ethanol content of their diet increased from 2 to 6%.[120] The biotin labelling approach was then used to recover damaged proteins from various liver fractions before the identity of the target protein and

adducting electrophile was determined via 2D-LC-MS/MS. Some 414 damaged proteins were identified, and while the study was hampered by problems of low sensitivity and adduct instability, four aldehydes were confirmed as contributors to protein carbonylation in ALD mice, namely 4-hydroxynonenal, malondialdehyde, 4-oxononenal and acrolein.[120] Network analysis of the protein dataset revealed a cluster of targets within metabolic pathways involved in hepatic lipid metabolism, including a number of acyl-CoA dehydrogenases that participate in the β-oxidation of lipids.[120] By identifying this pathway as a target for electrophilic LPO products, these experiments uncovered a novel explanation for the disruption of lipid metabolism and onset of steatosis during ALD, thereby illustrating how global technologies can bring new understandings to longstanding toxicological phenomena.

While these findings confirm that endogenous acrolein contributes to a toxic syndrome that accompanies intoxication with a popular xenobiotic, the nonspecificity of LPO cascades ensures that evaluation of its precise pathogenic role is complicated by the concurrent production of multiple endogenous electrophiles. Because the four lipid-derived electrophiles identified by Petersen and associates as mediators of protein damage in murine ALD share a conjugated α,β-unsaturated carbonyl system, they likely exert toxicity via a common ability to form adducts on thiol groups in target proteins.[2] Although concurrent exposure to multiple LPO-derived carbonyls could conceivably elicit additive or even synergistic toxicity due to a common underlying mechanism of action, few experimental studies have explored these possibilities. Intriguingly, recent characterisation of binary and ternary mixtures of various Type-2 alkenes by LoPachin and associates revealed the possibility of significant synergistic toxic interactions under some exposure

scenarios.[121] Although acrolein was not included within the Lopachin study, the possibility that it might enhance the toxicity of other endogenous LPO products, or that its own toxicity might be altered during simultaneous exposure to other Type-2 alkenes, deserves further attention. Taken together, these findings highlight that advances in the ability to capture carbonyl-adducted proteins from animal and human tissues can facilitate study of acrolein participation in toxic syndromes such as ALD, while also highlighting the need for closer attention to the toxicological interactions that might occur between structurally-related endogenous electrophiles during these complex syndromes.

4.4. Endogenous exposure as a pathophysiological response to tissue trauma

Complications accompanying physical injury to body tissues often afflict victims of automobile accidents, sporting injuries, firearm discharges, workplace mishaps and surgical interventions. Because oxidative stress usually features prominently in such syndromes due to the presence of ischemia-reperfusion injury and recruitment of activated phagocytic cells, induction of LPO has been associated with many forms of tissue trauma including pulmonary contusion,[122] skeletal muscle injury[123] and abdominal trauma.[124] In addition to physical trauma, a role for acrolein generation via LPO is emerging in other conditions that involve ischemia-reperfusion injury, such as myocardial infarction and retinal degeneration (Table 1). [125, 126] One anatomical setting in which LPO-derived acrolein likely plays a significant pathogenetic role is the spinal cord, a tissue which is highly vulnerable to traumatic injury since it is the conduit for sensory and autonomic communication between

the CNS and periphery. The rich fatty acid content of spinal cord tissue renders it highly susceptible to peroxidative chemistry following physical injury.

4.4.1. Spinal cord injury (SCI)

Due to the capacity for lasting disability, few injuries sustained by humans have more devastating consequences than those involving damage to spinal cord tissues (e.g. paraplegia, tetraplegia). SCI patients also endure many problems due to the diminished autonomic control of key body functions, leaving them vulnerable to recurring bladder and kidney infections, poor GI-tract activity, and cardiac and respiratory disturbances.[127] Human awareness of the devastating nature of SCI is long-standing; half a dozen spinal injuries sustained by workers on pyramid-building projects in Ancient Egypt are described in the famous Edwin Smith Papyrus that is dated to approx. 2,500 BC.[128] Sadly, improvements in the clinical management of SCI over the subsequent 4,000 years have been modest and while recent US studies suggest the incidence of such injuries has stabilized in recent decades, SCI is of rising significance in growing elderly cohorts due to their vulnerability to falls.[129]

SCI pathogenesis exhibits several distinct phases (**Scheme 7**) beginning with direct mechanical damage to the cord which typically elicits membrane shearing, blood vessel damage and oedema. The full clinical picture of SCI is unexplained by this initial damage, and a secondary spread of injury subsequently occurs centrifugally from the lesion site (**Scheme 7**).[130] The secondary phase features waves of necrotic and apoptotic cell death and involves deleterious biochemical changes that persist for days or even weeks. Such processes greatly expand the site of injury and culminate during the final chronic stage in

the formation of cysts comprising complex mixtures of astrocytes, Schwann cells, inflammatory cells and spared axons in various stages of myelination.[131] The chronic phase of SCI can last for months or years, and includes apoptosis, demyelination, glial scarring and Wallerian degeneration. Awareness that secondary processes greatly exacerbate SCI has inspired a large body of experimental work that evaluates various drug and biotechnology-based therapies. Strategies that limit cell death within the damaged spinal cord are particularly attractive since these may help preserve the functional capacity of the tissue.

Neuronal death following SCI proceeds via numerous mechanisms. A leading pathway involves axonal Ca^{2+} dyshomeostasis due to opening of voltage-dependent calcium channels, impairment of ATP-dependent Ca^{2+} efflux and disruption of intracellular Ca^{2+} sequestration by organelles. The resulting Ca^{2+} overload promotes neuronal death by inducing reactive nitrogen species production, mitochondrial damage, and protease or endonuclease activation.[132] A related neuropathic process involves the excitatory amino acid glutamate, the extracellular levels of which increase strongly after SCI. Glutamate excitotoxicity kills neuronal cells by activating AMPA receptors and eliciting depolarisation and osmotic stress, but also by exacerbating Ca^{2+} influx via voltage-dependent calcium channels.[133]

Oxidative stress also features strongly in SCI-associated neuronal death, with free radical formation occurring via multiple routes including reperfusion of ischemic tissue and xanthine oxidase activation; superoxide radical production during neurotransmitter redox-cycling; invasion and activation of neutrophils; mitochondrial uncoupling; and release of redox-active transition metals.[134] Free radicals are especially damaging to white matter

within the spinal cord which is rich in oxidation-prone lipids; activation of phospholipase A_2 occurs in the early stages of SCI, triggering a marked release of polyunsaturated lipids such as arachidonic acid.[135] The liberated lipid can be metabolised to various bioactive eicosanoids, but also undergoes nonenzymatic radical-catalysed fragmentation to generate noxious LPO products (e.g. malondialdehyde and 4-hydroxynonenal).[136,137] Due to their diffusible properties and chemical reactivity, reactive carbonyls are likely mediators of the secondary wave of tissue injury following SCI (**Scheme 7**).

A large body of work from the Shi laboratory strongly incriminates LPO-derived acrolein in the secondary phase of SCI.[138] In early work from this lab, an immunochemical strategy revealed a conspicuous increase in acrolein adducts within spinal cord proteins extracted from rats 4 h after compression injury, with protein damage peaking at 24 h and remaining elevated relative to controls 1 week post-injury.[139] Significantly, acrolein seemed to diffuse from its site of formation to induce damage in adjacent uninvolved spinal cord tissue, since acrolein-adducted proteins were detected not only in proteins extracted from the injury site (T10 to T11), but also in adjoining cord segments (T8-T9 and T12-T13). Comparable patterns were seen in the levels of 4-hydroxynonenal-modified proteins within the same spinal cord segments.[139] In related work, treating isolated guinea-pig spinal cord strips with acrolein elicited changes in axonal conduction that resemble those accompanying SCI.[140]

A recent *in vivo* rodent study conducted by Shi and associates boosts the case for acrolein involvement in SCI by showing that administration of acrolein directly into the spinal cord elicits prolonged behavioural and histological deficits resembling those accompanying SCI.[141] In this study, a microinjector was used to precisely administer

acrolein (0.1 or 1 μmol) into a defined site within the 10th thoracic level of the spinal cord of anaesthetised rats that were held in a stereotaxic apparatus. To minimise tissue injury during the injection procedure, acrolein was administered in a 1 μL volume of saline over a 5 min timeframe.[141] After recovery, the rats received regular behavioural testing that monitored their locomotor capacity over a 7-week period. Spinal cords recovered from rats sacrificed either 24 h or 7 weeks after acrolein administration were also subjected to histological and electron micrographic evaluation.[141] The findings revealed that a single administration of acrolein induced time- and dose-dependent declines in locomotor performance that were accompanied by persistent histological deficits including reactive gliosis, macrophage infiltration, neuronal degeneration and demyelination.[141] The tissue volume occupied by the lesion also expanded during the study, thereby suggesting that acrolein triggered cell death in neighbouring cord tissue. With the proviso that the injection of a bolus dose of acrolein may not fully reproduce the *in vivo* environment within traumatised tissue where ongoing LPO likely generates reactive carbonyls over an extended period, these results are intriguing and underscore a likely role for acrolein in SCI pathogenesis. Related work by Shi and associates showing that pharmacological intervention using compounds that suppress acrolein-mediated cell killing (e.g. hydralazine, phenelzine) afford tissue protection in rodent SCI models further reinforces this novel hypothesis.[142,143]

5. CONCLUDING THOUGHTS AND FUTURE DIRECTIONS

This Perspective has highlighted advances in our knowledge of the role of acrolein in human disease by clarifying four distinct exposure scenarios whereby the noxious

electrophile may participate in significant disease conditions (Table 1). For each syndrome highlighted, including those smoke-related conditions that can involve substantive exposure to exogenous acrolein, a recurring conclusion was that acrolein is likely only one of many toxic substances that contribute to the pathogenic process. Teasing out the actual contributions of a single species within these complex mixtures – and even one with such pronounced toxicological properties as those of acrolein – is likely to prove highly challenging.

Nonetheless, our survey has revealed genuine progress in the development of tools for evaluating acrolein involvement at distinct stages of the toxicology paradigm shown in **Scheme 2**. Such methods may well assist future efforts to strengthen the case for acrolein involvement in other disorders. For example, the emergence of sensitive LC-MS/MS assays for the quantitation of acrolein mercapturates within urine samples have certainly helped confirm its contribution to smoking-related disorders, but could also assist evaluation of the role of acrolein in other disease contexts. For example, these methods could be used to analyse urine samples from patients suffering from acute smoke intoxication (Section 4.1.2), cyclophosphamide bladder cystitis (Section 4.2.1), alcoholic liver disease (Section 4.3.1) or spinal cord injury (Section 4.4.1). Knowing how urinary levels of acrolein mercapturates vary during the onset and progression of such diseases, or between individuals suffering from conditions of different degrees of severity, may help clarify the extent of acrolein involvement in these conditions. The value of such investigations is heightened by recent findings from the Shi group which suggest that in a rodent model, urinary acrolein mercapturate (3HPMA) concentrations are more sensitive

indicators of blast-induced neurotrauma than conventional behavioural tests of locomotor behaviour and memory performance.[144]

Similarly, the work of Petersen and associates who optimised the use of biotin hydrazide techniques to identify acrolein-adducted proteins within the livers of ethanol-exposed rodents could assist the study of other diseases. Such techniques seem well suited to the characterisation of respiratory tract proteins that sustain damage by acrolein in tobacco smokers or SII victims, or the spinal cord proteins that are attacked by endogenous acrolein following physical trauma to the vertebral column. Analogous to the way in which pathways analysis helped identify β-oxidation pathway enzymes as targets for LPO-derived aldehydes in murine ALD, the use of systems toxicology approaches to analyse acrolein-adducted proteins in the smoke-exposed respiratory tract or damaged spinal cord could enrich our knowledge of these respective health conditions.

Our survey of recent literature has repeatedly highlighted the need for more clarity concerning the contributions of endogenous versus exogenous acrolein to particular diseases, but efforts are also needed to determine the actual endogenous sources of acrolein in distinct disorders. Although many studies have reported the presence of DNA and protein adducts in control subjects that were not knowingly exposed to exogenous acrolein, it is unknown whether these adducts form via the peroxidation of membrane lipids, amino acids such as threonine, or polyamines such as spermine (Section 2.4). The judicious use of mass spectrometry tools together with isotope-labelled precursors to these endogenous acrolein-donors within in vitro cellular or animal models could help resolve such uncertainty. Such knowledge might eventually enable the design of interventions to ablate endogenous acrolein production within particular diseases. Taken together, such

experiments could bring clarity to the ongoing effort to understand and mitigate the role of this highly noxious substance in major health disorders that take a continuing toll upon the individual and collective health of human societies.

References

1. Stevens, J.F. and Maier, C.S. (2008) Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. *Mol. Nutr. Food Res. 52,* 7-25.

2. LoPachin, R.M., Gavin, T., Petersen, D.R., and Barber, D.S. (2009) Molecular mechanisms of 4-hydroxy-2-nonenal and acrolein toxicity: nucleophilic targets and adduct formation. *Chem. Res. Toxicol. 22,* 1499-1508.

3. Burcham, P.C., Thompson, C.A., and Henry, P.J. (2010) Acrolein and the lung: chemical, molecular and pathological aspects, *Adv. Molec. Toxicol. 4,* 1-35.

4. Ghilarducci, D.P., and Tjeerdema, R. S. (1995) Fate and effects of acrolein. *Rev. Environ. Contam. Toxciol. 144,* 95–146.

5. Moghe, A., Ghare, S., Lamoreau, B., Mohammad, M., Barve, S., McClain, C., and Joshi-Barve, S. (2015) Molecular mechanisms of acrolein toxicity: relevance to human disease. *Toxicol Sci. 143,* 242-255.

6. Witmer, G.W. and Fagerstone, K.A. (2003) The use of toxicants in black-tailed prairie dog management: an overview, *Proceedings of the Wildlife Damage Management Conference, 10,* 359-369.

7. deCastro, R. B. (2014) Acrolein and asthma attack prevalence in a representative sample of the United States adult population 2000-2009. *PLoS One.* 9(5):e96926. doi: 10.1371/journal.pone.0096926.

8. Dost, F.N. (1991) Acute toxicology of components of vegetation smoke. *Rev. Environ. Contam. Toxicol.* 119, 1-46.

9. Burcham, P.C., Raso, A., and Thompson, C.A. (2010) Toxicity of smoke extracts towards A549 lung cells: role of acrolein and suppression by carbonyl scavengers. *Chem. Biol. Interact. 183,* 416-424.

10. Ewert, A., Granvogl, M., and Schieberle, P. (2014) Isotope-labeling studies on the formation pathway of acrolein during heat processing of oils. *J. Agric. Food Chem. 62,* 8524-8529.

11. Ho, S.S., Yu, J.Z., Chu, K.W. and Yeung, L.L.J. (2006) Carbonyl emissions from commercial cooking sources in Hong Kong. *Air Waste Manag. Assoc. 56,* 1091-1098.

12. Svendsen, K., Jensen, H.N., Sivertsen, I., and Sjaastad, A.K. (2002) Exposure to cooking fumes in restaurant kitchens in Norway. *Ann. Occup. Hyg. 46,* 395-400.

13. Seaman, V.Y., Bennett, D.H., and Cahill, T.M. (2009) Indoor acrolein emission and decay rates resulting from domestic cooking events. *Atmospheric Environment 43,* 6199–6204.

14. Hodson, E.C., Mailman, R.B. and Chambers, J.E. (Eds.) (1998) Acrolein, in *The Dictionary of Toxicology,* 2nd Ed., MacMillan, London, UK.

15. Hecht, S.S., Koh, W.P., Wang, R., Chen, M., Carmella, S.G., Murphy, S.E., and Yuan, J.M. (2015) Elevated levels of mercapturic acids of acrolein and crotonaldehyde in the urine of Chinese women in Singapore who regularly cook at home. *PLoS One* 10(3):e0120023. doi: 10.1371/journal.pone.0120023.

16. DeWoskin, R.S., Greenberg, M., Pepelko, W., and Strickland, J. (2003) Toxicological Review of Acrolein CAS No. 107-02-8, *US Environmental Protection Agency,* EPA/635/R-03/003: 1–106.

17. Eckert, E., Schmid, K., Schaller, B., Hiddemann-Koca, K., Drexler, H., and Göen, T. (2011) Mercapturic acids as metabolites of alkylating substances in urine samples of German inhabitants. *Int. J. Hyg. Environ. Health. 214,* 196-204.

18. Haussmann, H.J. (2012) Use of hazard indices for a theoretical evaluation of cigarette smoke composition. *Chem. Res. Toxicol. 25,* 794-810.

19. Burstyn, I. (2014) Peering through the mist: systematic review of what the chemistry of contaminants in electronic cigarettes tells us about health risks. *BMC Public Health. 14:18*. doi: 10.1186/1471-2458-14-18

20. Rahman, M.A., Hann, N., Wilson, A., and Worrall-Carter, L. (2014) Electronic cigarettes: patterns of use, health effects, use in smoking cessation and regulatory issues. *Tob. Induc. Dis. 12:21*. doi: 10.1186/1617-9625-12-21

21. McRobbie, H., Phillips, A., Goniewicz, M.L., Smith, K.M., Knight-West, O., Przulj, D., and Hajek, P. (2015) Effects of switching to electronic cigarettes with and without concurrent smoking on exposure to nicotine, carbon monoxide, and acrolein. *Cancer Prev. Res. (Phila). 8,* 873-878.

22. Goniewicz, M.L., Gawron, M., Smith, D.M., Peng, M., Jacob, P., and Benowitz, N.L. (2016) Exposure to Nicotine and Selected Toxicants in Cigarette Smokers Who Switched to Electronic Cigarettes: A Longitudinal Within-Subjects Observational Study. *Nicotine Tob. Res. pii*: ntw160.

23. Polosa, R. (2015) Electronic cigarette use and harm reversal: emerging evidence in the lung. *BMC Med. 13:54*. doi: 10.1186/s12916-015-0298-3.

24. Kaminskas, L.M., Pyke, S.M., and Burcham, P.C. (2004) Strong protein adduct trapping accompanies abolition of acrolein-mediated hepatotoxicity by hydralazine in mice. *J. Pharmacol. Exp. Ther. 310*, 1003-1010.

25. Toennes, S.W., Schmidt, K., Fandiño, A.S., and Kauert, G.F. (2002) A fatal human intoxication with the herbicide allyl alcohol (2-propen-1-ol). *J. Anal. Toxicol. 26*, 55-57.

26. Sanduja, R., Ansari, G.A., and Boor, P.J. (1989) 3-Hydroxypropylmercapturic acid: a biologic marker of exposure to allylic and related compounds. *J. Appl. Toxicol. 9*, 235-238.

27. Alarcon, R.A., Meienhofer, J., and Atherton, E. (1972) Isophosphamide as a new acrolein-producing antineoplastic isomer of cyclophosphamide. *Cancer Res. 32*, 2519-2523.

28. Giraud, B., Hebert, G., Deroussent, A., Veal, G.J., Vassal, G., and Paci, A. (2010) Oxazaphosphorines: new therapeutic strategies for an old class of drugs. *Expert Opin. Drug Metab. Toxicol. 6*, 919-938.

29. Ledauphin, J., Le Milbeau, C., Barillier, D., and Hennequin, D, (2010) Differences in the volatile compositions of French labelled brandies (Armagnac, Calvados, Cognac, and Mirabelle) using GC-MS and PLS-DA. *J. Agric. Food Chem. 58,* 7782-7793.

30. Bauer, R., Cowan, D.A., and Crouch, A. (2010) Acrolein in wine: importance of 3-hydroxypropionaldehyde and derivatives in production and detection. *J. Agric. Food Chem. 58*, 3243-3250.

31. Parent R.A., Caravello, H.E., and Sharp, D.E. (1996) Metabolism and distribution of [2,3-^{14}C]acrolein in Sprague-Dawley rats. *J. Appl. Toxicol. 16*, 449-457.

32. Watzek, N., Scherbl, D., Feld, J., Berger, F., Doroshyenko, O., Fuhr, U., Tomalik-Scharte, D., Baum, M., Eisenbrand, G., and Richling, E. (2012) Profiling of mercapturic acids of acrolein and acrylamide in human urine after consumption of potato crisps. *Mol. Nutr. Food Res. 56*, 1825-1837.

33. Anderson, M.M., Hazen, S.L., Hsu, F.F., and Heinecke, J.W. (1997) Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanal, and acrolein. A mechanism for the generation of highly reactive α-hydroxy and α,β-unsaturated aldehydes by phagocytes at sites of inflammation. *J. Clin. Invest. 99*, 424-432.

34. Pegg, A.E. (2013) Toxicity of polyamines and their metabolic products. *Chem. Res Toxicol. 26*, 1782-800.

35. Saiki, R., Park, H., Ishii, I., Yoshida, M., Nishimura, K., Toida, T., Tatsukawa, H., Kojima, S., Ikeguchi, Y., Pegg, A.E., Kashiwagi, K., and Igarashi, K. (2011) Brain infarction correlates more closely with acrolein than with reactive oxygen species. *Biochem. Biophys. Res. Commun. 404*, 1044-1049.

36. Sakata, K., Kashiwagi, K., Sharmin, S., Ueda, S., Irie, Y., Murotani, N., and Igarashi, K. (2003) Increase in putrescine, amine oxidase, and acrolein in plasma of renal failure patients. *Biochem. Biophys. Res. Commun. 305*, 143-149.

37. Uchida, K., Kanematsu, M., Sakai, K., Matsudak, T., Hattori, N., Mizuno, Y., Suzuki, D., Miyata, T., Noguchi, N., Niki, E., and Osawa, T. (1998) Protein-bound acrolein: potential markers for oxidative stress. *Proc. Natl. Acad. Sci. USA. 95*, 4882-4887.

38. Kehrer, J.P., and Klotz, L.O. (2015) Free radicals and related reactive species as mediators of tissue injury and disease: implications for Health. *Crit. Rev. Toxicol. 45*, 765-798.

39. Esterbauer, H., Schaur, R.J., and Zollner, H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med. 11*, 81-128.

40. Sleiman, M., Logue, J.M., Luo, W., Pankow, J.F., Gundel, L.A., and Destaillats, H. (2014) Inhalable constituents of thirdhand tobacco smoke: chemical characterization and health impact considerations. *Environ. Sci. Technol. 48,* 13093-13101.

41. Abraham, K., Andres, S., Palavinskas, R., Berg, K., Appel, K.E., and Lampen, A. (2011) Toxicology and risk assessment of acrolein in food. *Mol. Nutr. Food Res. 55,* 1277-1290.

42. Zamora, R., Aguilar, I., Granvogl, M., Hidalgo, F.J. (2016) Toxicologically relevant aldehydes produced during the frying process are trapped by food phenolics. *J. Agric. Food Chem. 64,* 5583-5589.

43. Kautiainen, A., Törnqvist, M., Svensson, K., and Osterman-Golkar, S. (1989) Adducts of malonaldehyde and a few other aldehydes to hemoglobin. *Carcinogenesis 10,* 2123-2130.

44. Colombo, G., Aldini, G., Orioli, M., Giustarini, D., Gornati, R., Rossi, R., Colombo, R., Carini, M., Milzani, A., and Dalle-Donne, I. (2010) Water-Soluble α,β-unsaturated aldehydes of cigarette smoke induce carbonylation of human serum albumin. *Antioxid. Redox Signal. 12,* 349-364.

45. Takamoto, S., Sakura, N., Namera, A., and Yashiki, M. (2004) Monitoring of urinary acrolein concentration in patients receiving cyclophosphamide and ifosphamide. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 806,* 59-63.

46. Al-Rawithi, S., El-Yazigi, A., Ernst, P., Al-Fiar, F., and Nicholls, P.J. (1998) Urinary excretion and pharmacokinetics of acrolein and its parent drug cyclophosphamide in bone marrow transplant patients. *Bone Marrow Transplant. 22,* 485-490.

47. Parent, R.A., Paust, D.E., Schrimpf, M.K., Talaat, R.E., Doane, R.A., Caravello, H.E., Lee, S.J., and Sharp, D.E. (1998) Metabolism and distribution of [2,3-14C]acrolein in Sprague-Dawley rats. II. Identification of urinary and fecal metabolites. *Toxicol. Sci. 43,* 110-120.

48. Mitchell, J.R., and Corcoran, G.B. (1977) Macromolecular binding in assessing drug and chemical-induced tissue lesions. *J. Environ. Pathol. Toxicol. 1,* 101-115.

49. Liebler, D.C. (2008) Protein damage by reactive electrophiles: targets and consequences. *Chem. Res. Toxicol. 21*, 117-128.

50. Burcham, P.C. (2013) *An Introduction to Toxicology*, Springer-Verlag, London.

51. LoPachin, R.M., and Gavin, T. (2016) Reactions of electrophiles with nucleophilic thiolate sites: relevance to pathophysiological mechanisms and remediation. *Free Radic. Res. 50*, 195-205.

52. Codreanu, S.G., Ullery, J.C., Zhu, J., Tallman, K.A., Beavers, W.N., Porter, N.A., Marnett, L.J., Zhang, B., and Liebler, D.C. (2014) Alkylation damage by lipid electrophiles targets functional protein systems. *Mol. Cell Proteomics 13*, 849-859.

53. Sturla, S.J., Boobis, A.R., FitzGerald, R.E., Hoeng‖, J., Kavlock, R.J., Schirmer, K., Whelan, M., Wilks, M.F., and Peitsch, M.C. (2014) Systems toxicology: From basic research to risk assessment. *Chem. Res. Toxicol. 27*, 314–329.

54. Corley, R.A., Kabilan, S., Kuprat, A.P., Carson, J.P., Jacob, R.E., Minard, K.R., Teeguarden, J.G., Timchalk, C., Pipavath, S., Glenny, R., and Einstein, D.R. (2015) Comparative risks of aldehyde constituents in cigarette smoke using transient computational fluid dynamics/Physiologically based pharmacokinetic models of the rat and human respiratory tracts. *Toxicol. Sci. 146*, 65-88.

55. Kutzman, R.S., Popenoe, E.A., Schmaeler, M., and Drew, R.T. (1985) Changes in rat lung structure and composition as a result of subchronic exposure to acrolein. *Toxicology 34*, 139-151.

56. Rabe, K.F., Hurd, S., Anzueto, A., Barnes, P.J., Buist, S.A., Calverley, P., Fukuchi, Y., Jenkins, C., Rodriguez-Roisin, R., van Weel, C., and Zielinski, J. (2007) Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am. J. Respir. Crit. Care Med. 176,* 532-555.

57. Buist, A.S., Vollmer, W.M., and McBurnie, M.A. (2008) Worldwide burden of COPD in high- and low-income countries. Part I. The burden of obstructive lung disease (BOLD) initiative. *Int. J. Tuberc. Lung Dis. 12*, 703-708.

58. Decramer, M., Janssens, W., and Miravitlles, M. (2012) Chronic obstructive pulmonary disease. *Lancet 379*, 1341-1351.

59. Fletcher, M.J., Upton, J., Taylor-Fishwick, J., Buist, S.A., Jenkins, C., Hutton, J., Barnes, N., Van Der Molen, T., Walsh, J.W., Jones, P., and Walker, S. (2011) COPD uncovered: an international survey on the impact of chronic obstructive pulmonary disease [COPD] on a working age population. *BMC Public Health 11*, 612.

60. Faes, K., De Frène, V., and Cohen, J., and Annemans, L. (2016) Resource use and healthcare costs of COPD patients at the end of life: A Systematic Review. *J. Pain Symptom Manage*. 8. pii: S0885-3924(16)30174-9. doi: 10.1016/j.jpainsymman.2016.04.007. [in press]

61. Jones, R.L., Noble, P.B., Elliot, J.G., and James, A.L. (2016) Airway remodelling in COPD: It's not asthma! *Respirology,* doi: 10.1111/resp.12841. [in press].

62. Sundar, I.K., Yao, H., and Rahman, I. (2013) Oxidative stress and chromatin remodelling in chronic obstructive pulmonary disease and smoking-related diseases. *Antioxid. Redox Signal. 18*, 1956-1971.

63. Yeager, R.P., Kushman, M., Chemerynski, S., Weil, R., Fu, X., White, M., Callahan-Lyon, P., and Rosenfeldt, H. (2016) Proposed mode of action for acrolein respiratory toxicity associated with inhaled tobacco smoke. *Toxicol. Sci. 151*, 347-364.

64. Lyon, J.P, Jenkins, L.J., Jones, R.A., Coon, R.A. and Siegel, J. (1970) Repeated and continuous exposure of laboratory animals to acrolein. *Toxicol. Appl. Pharmacol. 17,* 726–732.

65. Deshmukh, H.S. , Shaver, C., Case, L.M., Dietsch, M., Wesselkamper, S.C., Hardie, W.D., Korfhagen, T.R. Corradi, M., Nadel, J.A., Borchers, M.T., and Leikauf, G.D. (2008) Acrolein-activated matrix metalloproteinase 9 contributes to persistent mucin production. *Am. J. Respir. Cell Mol. Biol. 38*, 446–454.

66. Deshmukh, H.S., Case, L.M., Wesselkamper, S.C., Borchers, M.T., Martin, L.D., Shertzer, H.G., Nadel, J.A., and Leikauf, G.D. (2005) Metalloproteinases mediate mucin 5AC expression by epidermal growth factor receptor activation. *Am. J. Respir. Crit. Care Med. 171*, 305-314.

67. Deshmukh, H.S., McLachlan, A., Atkinson, J.J., Hardie, W.D., Korfhagen, T.R., Dietsch, M., Liu, Y., Di, P.Y., Wesselkamper, S.C., Borchers, M.T., and Leikauf,

G.D. (2009) Matrix metalloproteinase-14 mediates a phenotypic shift in the airways to increase mucin production. *Am. J. Respir. Crit. Care Med. 180*, 834-845.

68. Deshmukh, H.S., Shaver, C., Case, L.M., Dietsch, M., Wesselkamper, S.C., Hardie, W.D., Korfhagen, T.R., Corradi, M., Nadel, J.A., Borchers, M.T., and Leikauf, G.D. (2008) Acrolein-activated matrix metalloproteinase 9 contributes to persistent mucin production. *Am. J. Respir. Cell Mol. Biol. 38*, 446-454.

69. Kirkham, P.A., Spooner, G., Ffoulkes-Jones, C., and Calvez, R. (2003) Cigarette smoke triggers macrophage adhesion and activation: Role of lipid peroxidation products and scavenger receptor, *Free Radic. Biol. Med. 35*, 697–710.

70. 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., and Choi, A.M. (2004) Comprehensive gene expression profiles reveal pathways related to the pathogenesis of chronic obstructive pulmonary disease, *Proc. Natl. Acad. Sci. U. S. A. 101*, 14895–14900.

71. Thompson, C.A., and Burcham, P.C. (2008) Genome-wide transcriptional responses to acrolein. *Chem. Res. Toxicol. 21*, 2245-2256.

72. Reynolds, P.R., Cosio, M.G., and Hoidal, J.R. (2006) Cigarette smoke-induced Egr-1 upregulates proinflammatory cytokines in pulmonary epithelial cells. *Am. J. Respir. Cell Mol. Biol. 35*, 314–319.

73. Ning, W., Dong, Y., Sun, J., Li, C., Matthay, M.A., Feghali-Bostwick, C.A., and Choi, A.M. (2007) Cigarette smoke stimulates matrix metalloproteinase-2 activity via EGR-1 in human lung fibroblasts, *Am. J. Respir. Cell Mol. Biol. 36*, 480–490.

74. Conklin, D.J., Haberzettl, P., Prough, R.A. and Bhatnagar, A. (2009) Glutathione-S-transferase P protects against endothelial dysfunction induced by exposure to tobacco smoke. *Am. J Physiol. Heart Circ. Physiol. 296*, H1586-H1597.

75. Burcham, P.C., Raso, A., Thompson, C., and Tan, D. (2007) Intermolecular protein cross-linking during acrolein toxicity: efficacy of carbonyl scavengers as inhibitors of heat shock protein-90 cross-linking in A549 cells. *Chem. Res. Toxicol. 20*, 1629-1637.

76. Zhang, S., Villalta, P.W., Wang, M., and Hecht, S.S. (2007) Detection and quantitation of acrolein-derived 1,N2-propanodeoxyguanosine adducts in human lung by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Chem. Res. Toxicol. 20*, 565-571.

77. Kim, S.I., Pfeifer, G.P., and Besaratinia, A. (2007) Lack of mutagenicity of acrolein-induced DNA adducts in mouse and human cells. *Cancer Res. 67*, 11640-11647.

78. Wang, H.T., Zhang, S., Hu, Y., and Tang, M.S. (2007) Mutagenicity and sequence specificity of acrolein-DNA adducts. *Chem. Res. Toxicol. 22*, 511-517.

79. Squadrito, G.L., Cueto, R., Dellinger, B., and Pryor, W.A. (2001) Quinoid redox cycling as a mechanism for sustained free radical generation by inhaled airborne particulate matter. *Free Radic. Biol. Med. 31*, 1132-1138.

80. Comandini, A., Rogliani, P., Nunziata, A., Cazzola, M., Curradi, G., and Saltini, C. (2009) Biomarkers of lung damage associated with tobacco smoke in induced sputum. *Respir. Med. 103*, 1592-1613.

81. Rech, T.H., Boniatti, M.M., Franke, C.A., Lisboa, T., Wawrzeniak, I.C., Teixeira, C., Maccari, J.G., Schaich, F., Sauthier, A., Schifelbain, L.M., Riveiro, D.F., da Fonseca, D.L., Berto, P.P., Marques, L., Dos Santos, M.C., de Oliveira, V.M., Dornelles, C.F., and Vieira, S.R. (2016) Inhalation injury after exposure to indoor fire and smoke: The Brazilian disaster experience. *Burns 42*, 884-890.

82. Ryan, C.M., Schoenfeld, D.A., Thorpe, W.P., Sheridan, R.L., Cassem, E.H., and Tompkins, R.G. (1998) Objective estimates of the probability of death from burn injuries. *N. Engl. J. Med. 338*, 362-366.

83. Walker, P.F., Buehner, M. F., Wood, L.A., Boyer, N.L., Driscoll, I.R., Lundy, J.B., Cancio L.C., and Chung, K.K. (2015) Diagnosis and management of inhalation injury: an updated review. *Critical Care 19*, 351

84. Enkhbaatar, P., and Traber, D.L. (2004) Pathophysiology of acute lung injury in combined burn and smoke inhalation injury, *Clin. Sci. (Lond.)*, 107, 137–143.

85. Tanizaki, S. (2015) Assessing inhalation injury in the emergency room. *Open Access Emerg. Med. 7*, 31-37.

86. Matthay, M.A., Folkesson, H.G., and Clerici, C. (2002) Lung epithelial fluid transport and the resolution of pulmonary oedema. *Physiol. Rev. 82*, 569–600.

87. Fuchs, P.C. , Demir, E., Reuber, K., Stromps, P., Wolter, T., and Pallua, N. (2009) Intra-alveolar IL-6 levels following burn and inhalation injury. *Burns 35*, 840–844.

88. Stromps, J.P., Fuchs, P., Demir, E., Grieb, G., Reuber, K., and Pallua, N. (2015) Intraalveolar TNF-α in combined burn and inhalation injury compared with intraalveolar interleukin-6. *J Burn Care Res. 36(2)*:e55-61.

89. Zikria, B.A., Ferrer, J.M., and Floch, H.F. (1972) The chemical factors contributing to pulmonary damage in ‘‘smoke poisoning.’’ *Surgery 71*, 704–709.

90. Hales, C.A. Barkin, P.W. Jung, W., Trautman, E., Lamborghini, D., Herrig, N., and Burke, J. (1988) Synthetic smoke with acrolein but not HCl produces pulmonary edema. *J. Appl. Physiol. 64*, 1121–1133.

91. Efimova, O., Volokhov, A.B., Iliaifar, S., and Hales, C.A. (2000) Ligation of the bronchial artery in sheep attenuates early pulmonary changes following exposure to smoke, *J. Appl. Physiol. 88*, 888–893.

92. Hales, C.A., Musto, S.W. Janssens, S., Jung, W., Quinn, D.A., and Witten, M. (1992) Smoke aldehyde component influences pulmonary edema. *J. Appl. Physiol. 72*, 555–561.

93. Janssens, S.P., Musto, S.W., Hutchison, W.G., Spence, C., Witten, M., Jung, W., and Hales, C.A. (1985) Cyclooxygenase and lipoxygenase inhibition by BW-755C reduces acrolein smoke-induced acute lung injury. *J. Appl. Physiol. 77*, 888-895.

94. Burcham, P.C., Raso, A., and Thompson, C.A. (2010) Intermediate filament carbonylation during acute acrolein toxicity in A549 lung cells: functional consequences, chaperone redistribution, and protection by bisulfite. *Antioxid. Redox Signal. 12*, 337-347.

95. Jang, A.S., Concel, V.J., Bein, K., Brant, K.A., Liu, S., Pope-Varsalona, H., Dopico, R.A. Jr, Di, Y.P., Knoell, D.L., Barchowsky, A., and Leikauf, G.D. (2011)

Endothelial dysfunction and claudin 5 regulation during acrolein-induced lung injury. *Am. J. Respir. Cell Mol, Biol. 44,* 483-90.

96. Cox, P.J. (1979) Cyclophosphamide cystitis - identification of acrolein as the causative agent. *Biochem. Pharmacol. 28,* 2045-2049.

97. Conklin, D.J., Haberzettl, P., Lesgards, J.F., Prough, R.A., Srivastava, S., and Bhatnagar, A. (2009) Increased sensitivity of glutathione S-transferase P-null mice to cyclophosphamide-induced urinary bladder toxicity. *J. Pharmacol. Exp. Ther. 331*, 456-469.

98. Kim, S.H., Lee, I.C., Baek, H.S., Shin, I.S., Moon, C., Bae, C.S., Kim, S.H., Kim, J.C., and Kim, H.C. (2014) Mechanism for the protective effect of diallyl disulfide against cyclophosphamide acute urotoxicity in rats. *Food Chem. Toxicol. 64*, 110-118.

99. Schimmel, K.J., Richel, D.J., Van den Brink, R.B. and Guchelaar, H.J. (2004) Cardiotoxicity of cytotoxic drugs. *Cancer Treat. Rev. 30,* 181–191.

100. Mythili, Y., Sudharsan, P.T., Sudhahar, V., and Varalakshmi, P. (2006) Protective effect of DL-α-lipoic acid on cyclophosphamide induced hyperlipidemic cardiomyopathy. *Eur. J. Pharmacol. 543,* 92-96.

101. Arikketh, D., Niranjali, S., and Devaraj, H. (2004) Detection of acrolein-lysine adducts in plasma low-density lipoprotein and in aorta of cyclophosphamide-administered rats. *Arch Toxicol. 78,* 397-401.

102. Conklin, D.J., Haberzettl, P., Jagatheesan, G., Baba, S., Merchant, M.L., Prough, R.A., Williams, J.D., Prabhu, S.D., and Bhatnagar, A. (2015) Glutathione S-transferase P protects against cyclophosphamide-induced cardiotoxicity in mice. *Toxicol. Appl. Pharmacol. 285,* 136-148.

103. Alderton, A.L., Faustman, C., Liebler, D.C., and Hill, D.W. (2003) Induction of redox instability of bovine myoglobin by adduction with 4-hydroxy-2-nonenal. *Biochemistry* 42, 4398-4405.

104. Buhler, D.R. and Wang, J.-L. (2013) Allylamine. In *Ethel Browning's Toxicity and Metabolism of Industrial Solvents*, 2nd ed. Vol. 2: Nitrogen and Phosphorus Solvents. (Buhler, D.R., and Reed, D. J, Eds.); pp. 3- 10, Elsevier Science, Amsterdam.

105. Boor, P.J., Sanduja, R., Nelson, T.J., and Ansari, G.A. (1987) *In vivo* metabolism of the cardiovascular toxin, allylamine. *Biochem. Pharmacol. 36*, 4347-4353.

106. Boor, P.J., and Hysmith, R.M. (1987) Allylamine cardiovascular toxicity. *Toxicology 44*, 129-145.

107. Conklin, D.J., Bhatnagar, A., Cowley, H.R., Johnson, G.H., Wiechmann, R.J., Sayre, L.M., Trent, M.B., and Boor, P.J. (2006) Acrolein generation stimulates hypercontraction in isolated human blood vessels. *Toxicol. Appl. Pharmacol. 217*, 277-288.

108. Uchida, K., Kanematsu, M., Morimitsu, Y., Osawa, T., Noguchi, N., and Niki, E. (1998) Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. *J. Biol. Chem. 273*, 16058-16066.

109. Shao, B., Fu, X., McDonald, T.O., Green, P.S., Uchida, K., O'Brien, K.D., Oram, J.F., and Heinecke, J.W. (2005) Acrolein impairs ATP binding cassette transporter A1-dependent cholesterol export from cells through site-specific modification of apolipoprotein A-I. *J. Biol. Chem. 280*, 36386-36396.

110. Schwartz, J.M., and Reinus, J.F. (2012) Prevalence and natural history of alcoholic liver disease. *Clin. Liver Dis. 16*, 659-666.

111. Chacko, K.R., and Reinus, J. (2016) Spectrum of alcoholic liver disease. *Clin. Liver Dis.* 20, 419-427.

112. Deaciuc, I.V., Arteel, G.E., Peng, X., Hill, D.B., and McClain, C.J. (2004) Gene expression in the liver of rats fed alcohol by means of intragastric infusion. *Alcohol* 33, 17-30.

113. Torok, N.J. (2015) Update on Alcoholic Hepatitis. *Biomolecules 5*, 2978-2986.

114. Lucey, M.R., Mathurin, P., and Morgan, T.R. (2009) Alcoholic hepatitis. *N. Engl. J. Med. 360*, 2758-2769.

115. Sugimoto, K., and Takei, Y. (2016) Pathogenesis of alcoholic liver disease. *Hepatol. Res. (in press)* doi: 10.1111/hepr.12736.

116. Bailey, S.M., and Cunningham, C.C. (2002) Contribution of mitochondria to oxidative stress associated with alcoholic liver disease. *Free Radic. Biol. Med. 32*, 11-16.

117. Cederbaum, A.I. (2010) Role of CYP2E1 in ethanol-induced oxidant stress, fatty liver and hepatotoxicity. *Dig. Dis. 28*, 802-811.

118. Jiang, J.X., and Török, N.J. (2014) NADPH Oxidases in chronic liver diseases. *Adv. Hepatol*. DOI: 10.1155/2014/742931

119. Osna, N.A., Carter, W.G., Ganesan, M., Kirpich, I.A., McClain, C.J., Petersen, D.R., Shearn, C.T., Tomasi, M.L., and Kharbanda, K.K. (2016) Aberrant post-translational protein modifications in the pathogenesis of alcohol-induced liver injury. *World J. Gastroenterol. 22*, 6192-6200.

120. Galligan, J.J., Smathers, R.L., Fritz, K.S., Epperson, L.E., Hunter L.E., Petersen D.R. (2012) Protein carbonylation in a murine model for early alcoholic liver disease. *Chem. Res. Toxicol. 25*, 1012-1021.

121. Zhang, L., Geohagen, B.C., Gavin, T., and LoPachin, R.M. (2016) Joint toxic effects of the type-2 alkene electrophiles. *Chem. Biol. Interact. 254*, 198-206.

122. Aksua, B., Ayvazb, S., Aksuc, F., Karacad, T., Cemeke, M., Ayaze, A., and Demirtaşd, S. (2015) Effects of sphingosylphosphorylcholine against oxidative stress and acute lung ınjury ınduced by pulmonary contusion in rats. *J. Ped. Surg. 50*, 591–597.

123. Ghaly, A., and Marsh, D.R. (2010) Ischaemia-reperfusion modulates inflammation and fibrosis of skeletal muscle after contusion injury. *Int. J. Exp. Pathol. 91*, 244-255.

124. Rana, S.V., Kashinath, D., Singh, G., Pal, R., and Singh, R. (2006) Study on oxidative stress in patients with abdominal trauma. *Mol. Cell. Biochem. 291*, 161-166.

125. Conklin, D.J., Guo, Y., Jagatheesan, G., Kilfoil, P.J., Haberzettl, P., Hill, B.G., Baba, S.P., Guo, L., Wetzelberger, K., Obal, D., Rokosh, D.G., Prough, R.A., Prabhu, S.D., Velayutham, M., Zweier, J.L., Hoetker, J.D., Riggs, D.W., Srivastava, S., Bolli, R.,

and Bhatnagar, A. (2015) Genetic deficiency of glutathione S-transferase P increases myocardial sensitivity to ischemia-reperfusion injury. *Circ. Res. 117*, 437-449.

126. Chen, F.T., Yang, C.M., and Yang, C.H. (2013) The protective effects of the proteasome inhibitor bortezomib (velcade) on ischemia-reperfusion injury in the rat retina. *PLoS One* 8(5):e64262. doi: 10.1371/journal.pone.0064262.

127. Silva, N.A., Sousa, N., Reis, R.L., and Salgado, A.J. (2013) From basics to clinical: a comprehensive review on spinal cord injury. *Prog. Neurobiol. 114*, 25-57.

128. Holtz, A. and Levi, R. (2010) *Spinal Cord Injury*, Oxford University Press, New York Oxford.

129. Jain, N.B., Ayers, G.D., Peterson, E.N., Harris, M.B., Morse, L., O'Connor, K.C., and Garshick, E. (2015) Traumatic spinal cord injury in the United States, 1993-2012. *JAMA, 313*, 2236-2244.

130. Beattie, M.S. (2004) Inflammation and apoptosis: linked therapeutic targets in spinal cord injury. *Trends Mol. Med. 10*, 580-583.

131. Scwab, M.E. and Batholdi, D. (1996) Degeneration and regeneration of axons in the lesioned spinal cord. *Physiol. Rev., 76*, 319-370.

132. Chu, G.K.T., Tator, C.H., and Tymianski, M. (2000) Calcium and neuronal death in spinal neurons. Chapter 2 in *"Neurobiology of Spinal Cord Injury,"* Eds: Kalb, R.G. and Strittmatter, S.M., Humana Press: Totowa, N.J.

133. Profyris, C., Cheema, S.S., Zang, D., Azari, M.F., Boyle, K., and Petratos, S. (2004) Degenerative and regenerative mechanisms governing SCI. *Neurobiol. Dis. 15*, 415-436.

134. Fatima, G., Sharma, V.P., Das, S.K., and Mahdi, A.A. (2015) Oxidative stress and antioxidative parameters in patients with spinal cord injury: implications in the pathogenesis of disease. *Spinal Cord 53*, 3-6.

135. Toborek, M., Malecki, A., Garrido, R., Mattson, M.P., Hennig, B., and Young, B. (1999) Arachidonic acid-induced oxidative injury to cultured spinal cord neurons. *J. Neurochem. 73*, 684-692.

136. Qian, H. and Liu, D. (1997) The time course of malondialdehyde production following impact injury to rat spinal cord as measured by microdialysis and HPLC. *Neurochem. Res. 22*, 1231-1236.

137. Baldwin, S.A., Broderick, R., Osbourne, D., Waeg, G., Blades, D.A., and Scheff, S.W. (1998) The presence of 4-hydroxynonenal/protein complex as an indicator of oxidative stress after experimental spinal cord contusion in a rat model. *J. Neurosurg. 88*, 874-883.

138. Park, J., Muratori, B., and Shi, R. (2014) Acrolein as a novel therapeutic target for motor and sensory deficits in spinal cord injury. *Neural Regen. Res. 9*, 677-683.

139. Luo, J., Uchida, K., and Shi, R. (2005) Accumulation of acrolein-protein adducts after traumatic spinal cord injury. *Neurochem. Res. 30*, 291-295.

140. Shi, R., Luo, J., and Peasley, M. (2002) Acrolein inflicts axonal membrane disruption and conduction loss in isolated guinea-pig spinal cord. *Neuroscience 115*, 337-340.

141. Gianaris, A., Liu, N.K., Wang, X.F., Oakes, E., Brenia, J., Gianaris, T., Ruan, Y., Deng, L.X., Goetz, M., Vega-Alvarez, S., Lu, Q.B., Shi, R., and Xu, X.M. (2016) Unilateral microinjection of acrolein into thoracic spinal cord produces acute and chronic injury and functional deficits. *Neurosci. 326*, 84-94.

142. Chen, Z., Park, J., Butler, B., Acosta, G., Vega-Alvarez, S., Zheng, L., Tang, J., McCain, R., Zhang, W., Ouyang, Z., Cao, P., and Shi, R. (2016) Mitigation of sensory and motor deficits by acrolein scavenger phenelzine in a rat model of spinal cord contusive injury. *J. Neurochem. 138*, 328-338.

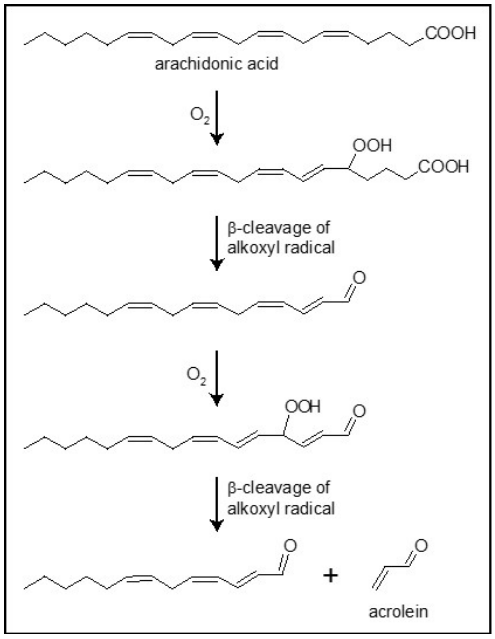
143. Park, J., Zheng, L., Marquis, A., Walls, M., Duerstock, B., Pond, A., Vega-Alvarez, S., Wang, H., Ouyang, Z., and Shi R. (2014) Neuroprotective role of hydralazine in rat spinal cord injury-attenuation of acrolein-mediated damage. *J. Neurochem. 129*, 339-349.

144. Walls, M.K., Race, N., Zheng, L., Vega-Alvarez, S.M., Acosta, G., Park, J., and Shi, R. (2016) Structural and biochemical abnormalities in the absence of acute deficits in mild primary blast-induced head trauma. *J. Neurosurg. 124*, 675-686.

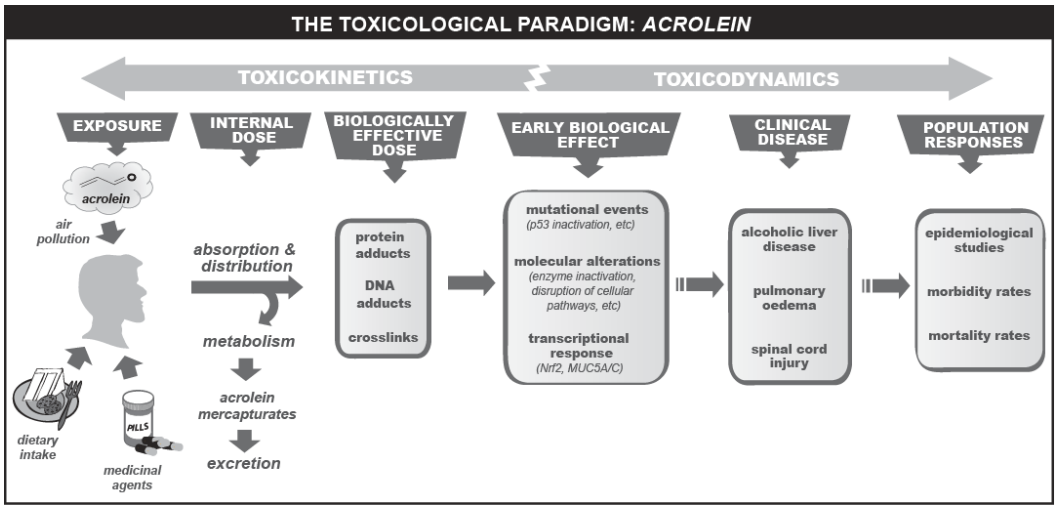
Bio: Phil Burcham obtained a Ph.D. in pharmacology before completing postdoctoral work in molecular toxicology at Vanderbilt. After a decade at the University of Adelaide, in 2005 he returned to the University of Western Australia where he teaches pharmacology and toxicology and conducts research on reactive smoke constituents. His fresher's course on pharmaceutical innovation, *PHAR1101: Drugs that Changed the World*, commenced in 2012 and soon became one of the most popular courses at his institution, attracting 780 enrolments in 2016. His *An Introduction to Toxicology* was published in 2014 by Springer (London, UK).

Table 1: Exposure scenarios whereby exogenous or endogenous acrolein can contribute to tissue injury in various diseases.

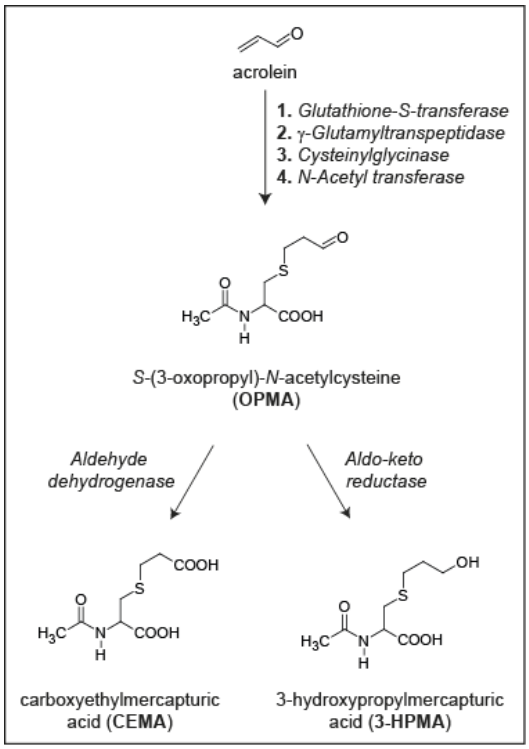
Exposure Scenario	Exposure Source or Pathophysiological Trigger	Human Disease
1. Acrolein exposure occurs primarily from exogenous sources	1) Cigarette smoke 2) Forest fire smoke	1) Chronic obstructive pulmonary disease (COPD) 2) Smoke inhalation injury (SII)
2. Acrolein forms as a by-product of xenobiotic biotransformation	1) Cyclophosphamide 2) Allyl compounds	1) Bladder, heart, lung toxicity 2) Hepatotoxicity (allyl alcohol), cardiotoxicity (allylamine)
3. Endogenous acrolein forms as a pathophysiological response to xenobiotic intoxication	1) Alcoholism	1) Alcoholic liver disease (ALD)
4. Endogenous acrolein forms as a pathophysiological response to tissue trauma	1) Ischemia-reperfusion injury 2) Neurodegeneration	1) Many – thrombotic myocardial infarction, peripheral artery disease, stroke, retinal injury 2) Spinal cord injury (SCI), Traumatic brain injury (TBI), Blast-induced neurotrauma (BINT)



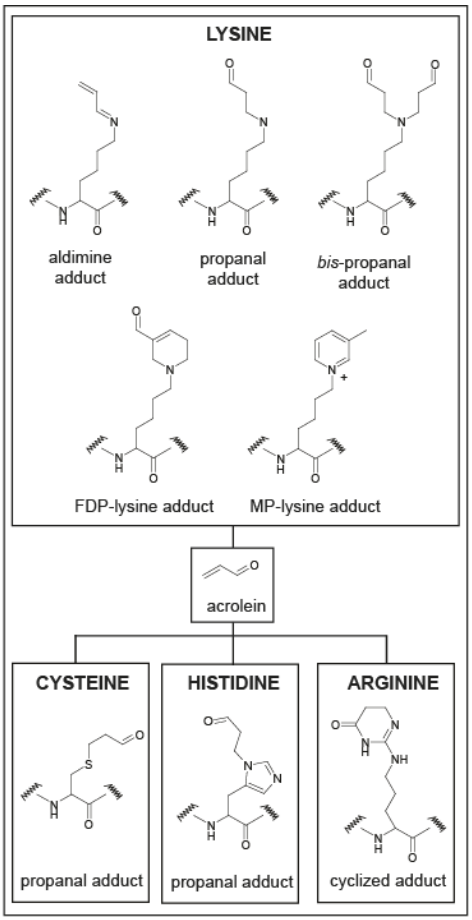
Scheme 1: Proposed route to acrolein formation during peroxidation of arachidonic acid.[1]



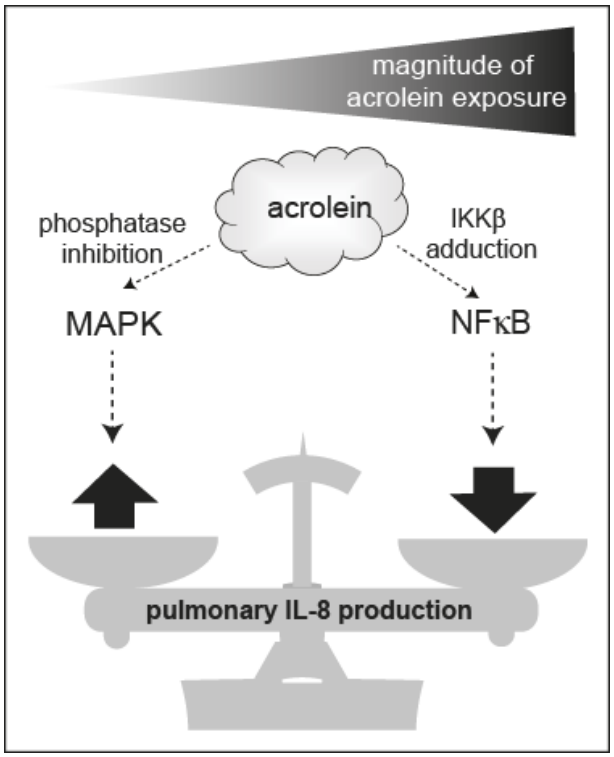
Scheme 2: Identifying valid biomarkers at each stage of the "Toxicological Paradigm" assists testing of associations between chemical exposures and clinically-relevant disease.



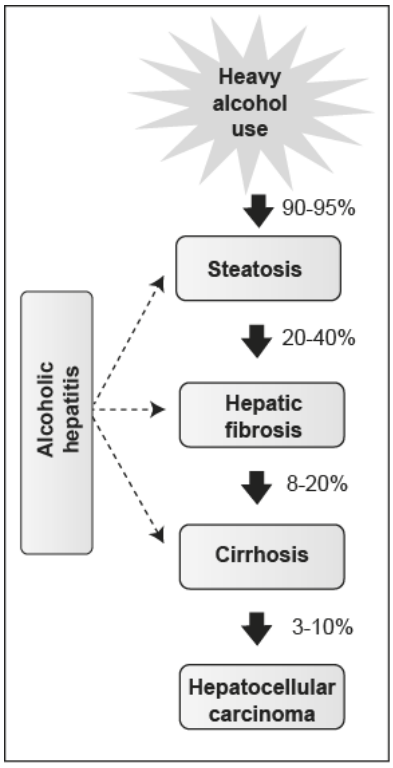
Scheme 3: The metabolism of acrolein produces the urinary mercapturates CEMA (*minor*) and 3-HPMA (*major*).[1]



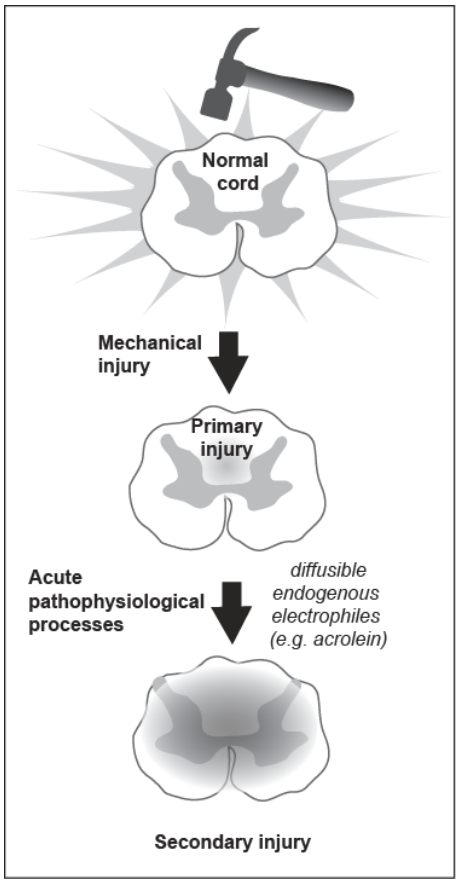
Scheme 4: The strong electrophilicity of acrolein results in a rich diversity of protein adducts. See Ref. 3 for details as well as information on DNA adducts and crosslinks.



Scheme 5: Acrolein likely exerts concentration-dependent opposing effects on the production of the pro-inflammatory cytokine IL-8 by lung epithelial cells. See ref. 3 for details.



Scheme 6: The progression of alcoholic liver disease involves a succession of overlapping stages. The percentages at each stage are taken from Ref. 99.



Scheme 7: The production of reactive endogenous electrophiles accompanies an expansion of the injured tissue following spinal cord injury (SCI). The secondary phase also involves expansion of the lesion longitudinally along the spinal cord (not shown).