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# Mechanisms of Soft and Hard Electrophile Toxicities

**Richard M. LoPachin, Ph.D.**[*,⊥], **Brian C. Geohagen, B.S.**[⊥], and **Lars U Nordstroem**[§]

[⊥]Department of Anesthesiology, Montefiore Medical Center, Albert Einstein College of Medicine, 111 E. 210th St, Bronx NY 10467

[§]The Chemical Synthesis & Biology Core Facility, Albert Einstein College of Medicine, Bronx, NY

## Abstract

Electron-deficient chemicals (electrophiles) react with compounds that have one or more unshared valence electron pairs (nucleophiles). The resulting covalent reactions between electrophiles and nucleophiles (e.g., Michael addition, $S_N2$ reactions) are important, not only to Organic Chemistry, but also to the fields of Molecular Biology and Toxicology. Specifically, covalent bond formation is the operational basis of many critically important cellular processes; e.g., enzyme function, neurotransmitter release, and membrane-vesicle fusion. Given this context it is understandable that these reactions are also relevant to Toxicology, since a significant number of xenobiotic chemicals are toxic electrophiles that can react with endogenous nucleophilic residues. Therefore, the purpose of this **Review** is to discuss electrophile-nucleophile chemistry as it pertains to cell injury and resulting organ toxicity. Our discussion will involve an introduction to the Hard and Soft, Acids and Bases (HSAB) theory of Pearson. The HSAB concept provides a framework for calculation of quantum chemical parameters that classify the electrophile and nucleophile covalent components according to their respective electronic nature (softness/hardness) and reactivity (electrophilicity/nucleophilicity). The calculated quantum indices in conjunction with corroborative *in vivo, in chemico* (cell free) and *in vitro* research can offer an illuminating approach to mechanistic discovery. Accordingly, we will provide examples that demonstrate how this approach has been used to discern mechanisms and sites of electrophile action.

### Keywords

nucleophiles; environmental pollution; toxicity; α,β-unsaturated aldehydes; type-2 alkenes; electrophilic toxicants

## 1.0. INTRODUCTION

**Electrophiles are electron-deficient chemicals that appear to be involved in toxicity through formation of covalent adducts via electron-rich biological nucleophiles.** Human

[*]Corresponding Author: Richard M. LoPachin, Ph.D., Department of Anesthesiology, Montefiore Medical Center, Albert Einstein College of Medicine, 111 E. 210th St, Bronx NY 10467, (718) 920-5054 (office phone), (914) 815-0590 (cell phone) richard.lopachin@einstein.yu.edu.

populations are exposed to a complex mixture of electrophilic toxicants derived from environmental, industrial, pharmaceutical and agricultural sources (e.g., 1, 4-naphthoquinone, acrolein, methyl mercury and chlorpyrophos oxon). This diverse exposure **could** represent potentially serious human health risk that is **possibly** complicated by synergistic or additive interactions among constituent electrophiles (e.g., Abraham et al., 2011; Bhatnagar, 2006; Bucham, 2016; Andrews and Clary, 1986; Bisesi, 1994; Dejamett et al., 2014; Faroon et al., 2008). Despite the **potential** risk of electrophile exposure, the mechanistic details of target selection and resulting cytotoxicity are not sufficiently understood. To address this information gap we used parameters derived from the hard and soft, acids and bases (HSAB) theory of Pearson to determine electronic disposition (softness, hardness) and reactivity of the electrophilic component (electrophilic index). In addition, we calculated the respective nucleophilic indices, which provide a measure of the propensity for an electrophile to react with a given nucleophile. Thus, in accordance with HSAB principles, we showed that electrophilic toxicants preferentially formed covalent adducts with nucleophiles of similar softness/hardness (see LoPachin et al, 2012). The toxicological relevance of these quantum mechanical parameters was established in corroborative *in chemico* and *in vivo* studies (e.g., see LoPachin et al., 2007a, b). Electrophilic toxicants can therefore be divided into groups according to their respective soft or hard demeanor and corresponding nucleophilic targets. Although electrophilic reactivity is the **important** determinant of toxic potency, the accuracy of this parameter is dependent upon intervening physicochemical variables that limit target accessibility; e.g., steric hindrance, solubility. Also to be considered in this Review, we will discuss the growing realization that toxic electrophiles do not target specific types of proteins or organelle. Instead, they cause toxicity by disabling protein constituents of an electrophile-receptive proteome. We propose that application of HSAB principles represents a rational basis for determining mechanisms of electrophile toxicity and for predicting the toxicity associated with new or unknown chemicals (see also; Anders, 2017; Schultz et al, 2006; Schwobel et al., 2011; Zhang et al., 2016). Understanding electrophile-based mechanisms of toxicity is also important for development of environmental remediation/avoidance strategies and for devising pharmacotherapeutic approaches to certain diseases. We will begin with a brief discussion of human exposure patterns to electrophilic chemicals that have multiple environmental and/or endogenous sources.

## 2.0. POSSIBLE SOURCES OF HUMAN EXPOSURE

Human populations are exposed to electrophilic chemicals derived from both anthropogenic (e.g., automobile exhaust, industrial pollution and drug-based toxicity) and natural sources (e.g., wood combustion, certain cheeses, cooking) and there is growing evidence that such exposures can have significant toxic consequences (Faroon et al., 2008; Stevens et al., 2008; Adams et al., 2008; Woodruff et al., 2007; Kumagai and Abiko, 2017; LoPachin and Gavin, 2014, 2012; Leikauf, 2002; Feron et al., 1991; Abraham et al., 2011 Andrews and Clary, 1986 ). For example, electrophiles such as methyl mercury (MeHg), formaldehyde, acrolein and methyl vinyl ketone (MVK) are pervasive contaminants of the ambient environment (air, water, soil; e.g., see Bisesi, 1994; O'Brien et al., 2005; Kehrer and Biswal; 2000). Many chemicals used in the manufacturing and agricultural industries are electrophiles (e.g., n-

propylbromide, vinyl chloride) or electrophile-producing protoxicants (e.g., n-hexane, chlorpyrifos) and therefore incidental exposure to these compounds represents a potential source of toxicity (Burcham, 2016; Samet and Wages, 2018; Kumagai and Abiko, 2017; LoPachin and DeCaprio, 2005; LoPachin and Gavin, 2015). Mainstream cigarette smoke, as well as second- and third-hand smoke, contain electrophiles from broad chemical classes; e.g., acrolein, acrylonitrile and cadmium (Bahl et al., 2016; Fujioka and Shibamoto, 2006; Werley et al., 2008). These constituents appear to be directly involved in the toxic consequences of smoking (Bahl et al., 2016; Llewellyn et al., 2009; van der Toorn et al., 2013). The therapeutic benefits of certain clinically important drugs (e.g., acetaminophen, cyclophosphamide, atorvastatin) are limited by biotransformation of the parent compounds to reactive electrophilic metabolites that subsequently produce toxicity; e.g., acetaminophen→N-acetyl-p-benzoquinone imine, cyclophosphamide →acrolein (Kalgutkar and Dalvie, 2015; Stachulski et al., 2012; Gurtoo et al., 1981). Cisplatin and other platinum (Pt)-based antineoplastic drugs (e.g., carboplatin, oxaliplatin) are highly effective and widely used in the treatment of solid tumors mainly of the testis, ovary, cervix, neck and bladder. Cisplatin chemotherapy is, however, frequently (40% - 50%) associated with a painful dose-dependent chemotherapy-induced peripheral neuropathy (CIPN; Seretny et al., 2014; Staff et al., 2017). This adverse outcome is likely mediated by the soft electrophilic character of platinum, which causes irreversible damage to sensory neurons in the dorsal root ganglion (Qing et al., 1996; Wang et al., 1996).

Growing evidence indicates that acrolein, 4-hydroxy-2-nonenal (HNE) and other unsaturated aldehyde electrophiles mediate the oxidative stress-induced pathogenic processes that appear to underlie many disease and tissue injury states; e.g., Alzheimer’s disease, atherosclerosis, diabetes and spinal cord injury (reviewed in Csala et al., 2015; LoPachin et al., 2008a, 2009a; Moghe et al., 2015; O’Brien et al., 2005; Shi et al., 2011). In this regard, research also suggests that inflammatory responses which often accompany pathogenic processes are mediated by endogenous electrophiles; e.g., acrolein, crotonaldehyde, acetaldehyde (Noerager et al., 2015; van der Toorn et al., 2013; Yin et al., 2015). Additional research indicates that environmentally-derived electrophilic aldehydes (e.g., acrolein, crotonaldehyde) with similar mechanisms of toxicity might act additively or synergistically with their endogenous counterparts to accelerate the onset and development of certain diseases (e.g., see Conklin et al., 2010; Wang et al., 2008; Dejarnett et al., 2015; Luo et al., 2007). Thus, humans are exposed to electrophilic toxicants through contact with diverse sources; e.g., atmospheric, personal, pharmaceutical and occupational environments. The possibility that environmental and endogenous electrophiles might interact to augment toxicity is a significant concern (see ahead).

## 3.0. TOXICOLOGICAL CONSEQUENCES OF COVALENT REACTIONS.

The formation of drug-receptor complexes in pharmacology involves short distance forces such as hydrogen-bonding, hydrophobic or van der Waals interactions. Reversible drug-receptor occupancy alters activity of the respective signal-transduction pathway, which subsequently initiates a change in cell physiology. In contrast, many toxic chemicals and/or their active metabolites exhibit electron deficient centers and are therefore classified as electrophiles (electron seeking). These chemicals form covalent bonds (e.g., 1,4-Michael

addition) with electron rich nucleophilic sites; e.g., the sulfhydryl thiolate state of cysteine, ε-amino group of lysine or the $N^2$ nitrogen of deoxyguanosine. Exposure of biological systems to electrophiles can cause cytotoxicity since the formation of covalently bonded adducts with specific nucleophilic sites can irreversibly disable the functions of enzymes, DNA, cytoskeletal proteins and other biological macromolecules (Fig. 1). Thus, for example, abundant evidence now indicates that α,β-unsaturated carbonyl derivatives of the type-2 alkene chemical class (e.g., acrolein, acrylamide, 4-hydroxy-2-nonenal) cause toxicity by forming Michael adducts with anionic sulfhydryl thiolate sites in the active zones of many cysteine-regulated enzymes (e.g., see Doorn and Petersen, 2002, 2003; Eliuk et al., 2007; Fritz et al., 2011, 2013; LoPachin et al., 2009a; Martyniuk et al., 2011; Seiner et al., 2007).

Covalent electrophile-nucleophile reactions are not arbitrary and are instead relatively selective as predicted by the HSAB theory of Pearson (1990). According to HSAB principles, electrophilic and nucleophilic species are classified as either “soft” or “hard” based on polarizability or the ease with which corresponding electron density can be delocalized to form a covalent bond. Remote electrons that are less influenced by the nucleus or those that occupy a larger volume (cloud) are more readily displaced into new bonding patterns. For example, the type-2 alkenes are designated as soft electrophiles because the delocalized π electrons are mobile. The corresponding electron cloud is stretched over four nuclear centers (C=C-C=0) based on the orbital interactions of the electron-withdrawing carbonyl group and the alkene moiety. As a consequence, the extended electron cloud is easily distorted and is by definition soft (polarizble). In contrast, hard electrophilic toxicants (e.g., 2,5-hexanedione, chlorpyrifos, and vinyl chloride) have highly localized non-extended charge densities at specific electron deficient centers. These chemicals are therefore characterized by low electron polarizability.

Nucleophiles are also designated as either soft or hard based on the polarizability of corresponding frontier shell electrons. Elements with large atomic radii such as sulfur have outer-shell electrons that are relatively far from the nucleus and are consequently highly polarizable. Thiol ionization (i.e., $SH \rightarrow S^-$) and the consequential expansion of the anionic cloud yield the relatively soft (easily polarizable) sulfhydryl thiolate nucleophile. In contrast, nitrogen and oxygen nucleophiles have relatively small atomic radii and, accordingly, their electron clouds are less susceptible to distortion. Such atoms are therefore harder nucleophiles; e.g., the $N^2$ nitrogen of deoxyguanosine.

## 4.0. QUANTITATIVE HSAB DESCRIPTORS OF COVALENT REACTIONS.

Covalent bond formation between reacting chemicals involves the electronic properties of the respective outermost orbitals. Consequently, the most important orbitals are the highest energy orbital that contains electrons (HOMO = Highest Occupied Molecular Orbital) and the lowest energy orbital that is vacant (LUMO = Lowest Unoccupied Molecular Orbital). The formation of a covalent adduct can be described as the overlap of the respective frontier orbitals and the transfer of electron density from the donating HOMO of the nucleophile to the recipient LUMO of the electrophile. The respective energies of the frontier molecular orbitals ($E_{LUMO}$ and $E_{HOMO}$) are known and can be used to calculate corresponding hardness ($\eta = [E_{LUMO} - E_{HOMO}]/2$) and softness ($\sigma = 1/\eta$). Within this context, softness is

an index of the relative ease with which electron density is transferred from the nucleophile to the electrophile during covalent bond formation. This parameter is related to the rate of the adduct-forming reaction. Finally, values of σ and η can be combined with other HSAB descriptors to estimate the propensity of an electrophile to undergo an adduct reaction. Specifically, the electrophilic index (ω) is a comprehensive measure of electrophilicity that combines softness and chemical potential (μ): $\omega = \frac{1}{2} \sigma\mu^2$. The latter parameter ($\mu = [E_{LUMO} + E_{HOMO}] / 2$) represents the ability of an electrophilic or nucleophilic species to undergo chemical change. Calculations of electrophilicity can provide quantitative information about the transition state energies involved in toxicant-protein covalent bond formation. Thus, values for ω correspond to the rate constant (k) of these adduct reactions and, as a consequence, are directly related to toxicant potency (see LoPachin et al., 2007a,b; 2009a). With respect to the nucleophile, the corresponding molecular orbital energies can also be used to calculate nucleophilic softness and chemical potential. In addition, the likelihood that a given nucleophile (A) will form an adduct with a given electrophile (B) can be predicted by calculating a nucleophilicity index ($\omega^-$); $\omega^- = \eta_A (\mu_A - \mu_B)^2/2(\eta_A + \eta_B)^2$. This parameter considers the hardness (η) and chemical potential (μ) of both the electrophilic and nucleophilic reactants. The electrophilic (ω) and nucleophilic ($\omega^-$) indices have been demonstrated to be reliable descriptors for a variety of electrophile-nucleophile interactions (LoPachin and Gavin 2012; LoPachin et al., 2008; 2012).

## 5.0. HSAB PRINCIPLES: PROVIDING MECHANISTIC INSIGHT INTO ELECTROPHILE TOXICITY

The HSAB model stipulates that toxic electrophiles will react preferentially with nucleophilic biological targets of comparable softness or hardness. Thus, for example, the conjugated α,β-unsaturated carbonyl structure of acrylamide (ACR), methyl vinyl ketone (MVK) and other type-2 alkenes is a soft electrophile that forms Michael-type adducts via second-order addition reactions with soft nucleophilic side chains of peptide amino acids (Table 1). Results from detailed studies suggest that unsaturated alkenes react faster with cysteine sulfhydryl groups than with respective primary and secondary nitrogen nucleophiles on lysine ε-amino groups and imidazole side chains of histidine. These kinetic differences indicate that cysteine residues are the preferred sites of type-2 alkene adduct formation; e.g., see Cai et al., 2009; Doorn and Petersen, 2003; LoPachin et al., 2007a,b; Martyniuk et al., 2011. However, it is important to recognize that not all cysteine sulfhydryl groups are functionally relevant and, therefore, it cannot be assumed that covalent adduction of these residues has toxicological significance (LoPachin and Barber, 2006). Substantial evidence, nonetheless, indicates that type-2 alkenes and other electrophiles; e.g., the ortho-quinone metabolite of dopamine; the acetaminophen metabolite, N-acetyl-p-benzoquinone imine (NAPQI) cause cytotoxicity via a common molecular mechanism involving the formation of irreversible adducts at specific cysteine residues (e.g., Cys280 of sirtuin 3; Cys152 of glyceraldehyde 3-phosphate dehydrogenase (GAPDH); Cys374 of actin; Dalle-Donne et al., 2007; Fritz et al., 2011; Leeming et al., 2015; Martyniuk et al., 2011). At physiological conditions (pH = 7.4), cysteine sulfhydryl groups exist mostly in the **weakly** nucleophilic thiol (0) state (Table 2) and are therefore not kinetically favorable targets for soft type-2 alkene electrophiles. Basic research complemented by calculations of HSAB parameters

have demonstrated that amino acid nitrogen groups (histidine, lysine) are hard, relatively weak nucleophiles and therefore are unfavorable targets for soft electrophiles (Barber and LoPachin, 2004; Barber et al., 2007; LoPachin et al., 2007a,b; LoPachin et al., 2009). Ionization of cysteine sulfhydryl groups, in contrast, yields the anionic thiolate (−1). As reflected in the corresponding higher $\omega^-$ values, this is a soft, highly nucleophilic state that reacts correspondingly faster with soft type-2 alkene electrophiles (Table 2). It is noteworthy that as a second-order reaction, the relative rates ($k_2$) of these soft-soft covalent adduct reactions will also vary as a function of the inherent reactivity (electrophilicity) of the electrophile component.

With respect to hard-hard interactions, the neurotoxic n-hexane metabolite, 2,5-hexanedione (2,5-HD), is a hard electrophile (Table 2) that preferentially forms 2,5-dimethyl-pyrrole adducts with hard nucleophilic nitrogen atoms of the ε-amino groups on lysine residues of neurofilaments and other cytoskeletal proteins. These aberrant proteins populated the characteristic giant axonal swellings in distal peripheral nerves of intoxicated humans and laboratory animals. The swellings were presumed to be responsible for the γ-diketone axonopathy associated with subchronic occupational exposure to n-hexane (DeCaprio et al., 1997; Graham et al., 1991). Accordingly, previous studies (reviewed in LoPachin and DeCaprio, 2004, 2005) demonstrated the presence of abundant high molecular weight neurofilament derivatives in nervous tissue preparations from 2,5-HD-intoxicated animals. However, more recent research has shown that these abnormal proteins were common to nervous tissue samples from both control and 2,5-HD intoxicated animals. As a result, the corresponding pathognomonic relevance is uncertain. Subsequent studies (Zhang et al., 2010) indicated that 2,5-HD selectively impaired the binding of microtubule-associated proteins (MAPs) to microtubules through adduction of lysine residues that mediate these protein-protein interactions. The critical role of MAPs in cytoskeletal structure and function suggests that these lysine residues are toxicologically relevant targets for 2,5-HD. **Hard-hard reactions also play a critical role in the antineoplastic mechanism of platinum (Pt)-based chemotherapy. Specifically, Pt has hard electrophilic attributes that can form adducts with hard nucleophilic residues on DNA (e.g., guanine N7). Pt binding of DNA disrupts transcription which leads to cancer cell death** (Wang et al., 1996).

## 6.0. THE CYSTEINE-CENTERED CATALYTIC TRIAD: A MOLECULAR TARGET FOR SOFT ELECTROPHILE TOXICITY

Our discussion has thus far indicated that the soft sulfhydryl thiolate state is the preferential target for the type-2 alkenes and other soft electrophiles. However, at the intracellular pH range (7.0-7.4), sulfhydryl groups exist largely in the non-reactive thiol (0) state. What therefore is the molecular condition that creates an available thiolate protein target? Highly nucleophilic sulfhydryl thiolate groups are found in cysteine-centered catalytic triads and other microenvironments that lower side-chain pKa values (discussed in LoPachin and Gavin, 2012; LoPachin et al., 2009; LoPachin and Barber, 2006). The aforementioned selective binding of soft electrophiles to specific cysteines suggests that these residues exist in a pKa lowering microenvironment. Research has revealed that these specialized amino acid configurations are located in the enzyme active zones where the nucleophilicity of the

thiolate is involved in protein function. For example, GAPDH catalyzes the conversion of glyceraldehyde 3-phosphate (G3P) to D-glycerate 1,3-bisphosphate. The initial step in this process is dependent upon Cys152 which mediates nucleophilic attack on the G3P carbonyl to yield a hemithioacetal intermediate. Normally, this reaction is regulated by reversible binding of redox modulators, e.g., nitric oxide (NO), hydrogen peroxide ($H_2O_2$), at thiolate acceptors of enzyme catalytic triads. In this regard, Cys152 of GAPDH is a thiolate-based nitric oxide (NO) acceptor (Mohr et al., 1994). More broadly, NO signaling regulates synaptic strength by modulating the activity of proteins involved in the synaptic vesicle cycle; e.g., NEM-sensitive factor (neurotransmitter release), the dopamine-transporter (re-uptake) and the vesicular monoamine transporter (vesicular monoamine transporter; Kiss 2000; Rudkouskaya et al., 2010). Our research suggested that these and other synaptic proteins were inactivated by ACR and that this leads to a disruption of neurotransmission at CNS synapses (Barber et al., 2004, 2007; LoPachin et al., 2004, 2007a,b, 2009). Since the ionization states of cysteine residues in catalytic triads play a direct role in enzyme function, irreversible acceptor adduction by ACR and other soft electrophiles will block redox signaling and directly inhibit enzyme function. Depending on the proteome affected (see ahead), this can disable broad cytophysiological processes thereby leading to cell damage and toxicity. It is important to note that electrophiles discriminate targets based on the favorability of the corresponding covalent reaction. This favorability is determined by the kinetics of the reaction which includes the relative reactivities (ω; $\omega^-$) of the electrophile and nucleophile components.

## 7.0. THE CELLULAR ELECTROPHILE-RESPONSIVE PROTEOME

The research discussed thus far indicates that the toxicology of soft electrophiles involves formation of irreversible adducts with soft nucleophilic thiolate groups located in active zones of many proteins. There is now evidence that electrophile-induced toxicity is mediated by the inactivation of multiple protein types by a given electrophile (e.g., see Barber et al., 2007; Barber and LoPachin, 2004). The size of the affected proteome is determined by the relative electrophilicity (ω) of the toxicant. Accordingly, a highly reactive electrophile (large ω) will form adducts with a board range of nucleophilic targets that vary with respect to nucleophilicity ($\omega^-$). In contrast, toxicants with lower electrophilicity (ω) will react preferentially with those target sites exhibiting higher nucleophilicity. The restricted availability of reactive targets reduces the size of the responsive proteome (see Martyniuk et al., 2011). The sensitive proteins are collectively known as an electrophile-responsive proteome (Ceaser et al., 2004; Higdon et al., 2012). Also contributing to the proteome size are additional physicochemical attributes such as active site accessibility, pKa and turnover of the constituent proteins. In essence, the proteome vulnerability serves to amplify the toxicological consequences of individual protein dysfunction.

Our research (Barber et al., 2007; Barber and LoPachin, 2004) identified a presynaptic ACR-sensitive proteome that included adduct formation with cysteine-regulated proteins mediating: 1) synaptic vesicle cycling; 2) mitochondrial/glycolytic energy production and 3) protein degradation. When considered *in toto*, the distribution of prote6in adducts clearly incorporated the respective proteomes for synaptic vesicles and presynaptic active zone (Burre and Volknandt, 2007; Morciano et al., 2009). Accordingly, our functional studies

indicated that unsaturated carbonyl derivatives (e.g., ACR, acrolein and HNE) had broad inhibitory effects on presynaptic vesicle cycling (Barber et al., 2004; LoPachin et al., 2004, 2006, 2007a,b, 2009) that corresponded to the onset and development of neurological deficits. That soft electrophiles react with soft nucleophilic thiolate sites on proteins within a given proteome appears to be a general mechanistic pattern. For example, the severe liver damage associated with acetaminophen (Tylenol™) overdose is mediated by the highly reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI). This soft electrophile causes hepatocyte injury by depleting glutathione (GSH) and by forming Michael adducts with soft nucleophilic thiolate sites on liver cell proteins. Research suggests that the NAPQI-sensitive proteome incorporates specific cysteine-directed proteins from a number of subcellular organelles, pathways and regions; e.g., cytoplasm, mitochondria (Dietze et al., 1997; Hoffmann et al., 1985; Leeming et al., 2015; Reid et al., 2005). Therefore, putative mechanisms of toxicity should incorporate the concept of electrophile (soft or hard)-induced damage to responsive cellular proteomes.

Alternatively, the proteome can be narrowly populated with respect to nucleophile target diversity. For example, the organophosphate insecticide, chlorpyrifos (Cpf), is metabolized to chlorpyrifos oxon (Cpo), a highly reactive hard electrophile. Acetyl cholinesterase (AChE) activity is selectively inhibited by Cpo adduction of serine oxyanion, a hard nucleophile located at the terminus of the AChE gorge. Because this enzyme is responsible for acetylcholine (ACh) metabolism, the irreversible inhibition of this enzyme causes cholinergic neurotoxicity through an increase in synaptic acetylcholine. Cpo can selectively gain access to the nucleophilic terminus because it exhibits many of the spatial and electronic characteristics of ACh and accordingly, interacts with AChE as an ACh analogue (Ripoll et al, 1993).

## 8.0. PHYSICOCHEMICAL AND CELLULAR FEATURES THAT INFLUENCE SOFT ELECTROPHILE TOXICITY

Although the relative electrophilicity (ω) of a chemical is an important determinant of corresponding toxicity, it cannot be assumed that this chemical will carry the risk of toxicity since other physicochemical, toxicokinetic and cellular features can influence the covalent reaction with a nucleophile. Thus, for example, in previous studies of toxic unsaturated carbonyl derivatives (LoPachin et al., 2008a,b), we found that the corresponding electrophilicity (ω) and softness (σ) values for 4-hydroxy-2-nonenal (HNE) exceeded those for acrolein or methyl vinyl ketone (MVK; Table 1). This suggested that HNE was a more significant toxicant than either acrolein or MVK. However, when the respective toxic potencies ($IC_{50}$ of synaptosomal sulfur depletion) and adduct rate constants ($k_2$) were determined as indices of actual toxicity, the HNE values were significantly lower than predicated by the aforementioned HSAB calculations (Table 1; LoPachin et al., 2009a,b; LoPachin and Gavin, 2014). Whereas this inconsistency might suggest that ω and σ cannot accurately predict chemical reactivity and therefore toxicity, the extended alkane tail of HNE could impede access to the active sites of many enzymes. **In this regard, the reduced toxicity might be caused by unfavorable reaction kinetics that could arise from steric hindrance.**

**Also influencing the onset and development of toxicity is the induction of glutathione transferase (GST), a Phase II enzyme that catalyzes GSH-electrophile conjugation.** Other physicochemical attributes such as solubility and acid-base equilibrium can also influence the correspondence between experimentally derived electrophile behavior and that expected based on HSAB calculations (LoPachin et al., 2012; LoPachin and Gavin, 2014). These types of disagreements are expected since the HSAB algorithms do not incorporate these physicochemical and toxicokinetic properties. Nonetheless, results can be correctly interpreted since these mitigating physicochemical traits can be recognized by their structural characteristics; e.g., compare the structural differences that characterize acrolein (non-hindered), citral (hindered) and crotonaldehyde (partially hindered; LoPachin and Gavin, 2014).

In addition to the aforementioned physicochemical and toxicokinetic features, certain cell-based characteristics can also shape electrophile toxicity. In this regard, ACR, methyl acrylate (MA), ethyl methacrylate (EMA) are unlikely toxicants given their low electrophilic reactivity and consequential slow adduct formation (Table 2). Nonetheless, we showed that ACR intoxication was associated with nerve terminal dysfunction and eventual degeneration in rat brain and spinal cord (LoPachin et al., 2003; LoPachin and Gavin, 2012). Animals Intoxicated over a very broad ACR exposure-range (1.0-50 mg/kg/d) expressed hindlimb skeletal muscle weakness, decreased grip strength, gait incoordination and weight loss (LoPachin et al., 2002). Early studies revealed that neurotoxicity was a selective effect of ACR since other organ toxicity (e.g., liver, kidney) were not identified (LoPachin et al., 2003).

The presynaptic focus revealed in our studies did not appear to be due to ACR targeting of nerve terminals, rather it involved the influence of cell-specific features that predispose nerve terminals to soft electrophile toxicity. Thus, ACR is a water-soluble unsaturated alkene with a large volume of distribution that includes the CNS (Barber et al., 2001). Presynaptic neurotransmission is a complex process that is highly vulnerable to electrophile attack since it involves the coordinated function of multiple NO/cysteine-regulated proteins (LoPachin et al., 2003; 2008; LoPachin and Barber, 2006; LoPachin and Gavin, 2012, 2014; 2015). The nerve terminal is therefore a target rich environment for soft electrophiles. This nerve region is also anatomically separated from the cell body and is therefore devoid of transcriptional or translational capabilities. As a consequence, this distal region is limited with respect to mounting transcription-based reparative or protective reactions; e.g., the Nrf2-Keap1 antioxidant response (Zhang et al., 2011). In the absence of synthetic processes, the nerve terminal proteome must be maintained by perikaryal protein synthesis and subsequent anterograde transport. The turnover rates of many presynaptic proteins are slow (Calakos and Scheller, 1996; Katyare and Shallom, 1988), presumably as an attempt to limit cell body stress through reduced material expenditure and increased efficiency. However, research indicates that because of slow turnover rates proteins inactivated through cysteine adduct formation are replaced slowly leading to a gradual deficit of normal functioning proteins. In this regard, proteomic analyses (Barber and LoPachin, 2004; Barber et al., 2007) have shown that presynaptic cysteine adducts build-up in correspondence with the cumulative development of ACR neurological symptoms.

## 9.0. SUMMARY

Toxic electrophilic chemicals are an endemic component of the human biosphere. Specifically, humans are exposed to complex mixtures of soft and hard electrophiles that are derived from a variety of anthropogenic and natural sources. It is likely that this diverse exposure carries significant health risks and possibly augments development of disease processes that are mediated by endogenous electrophiles. Yet, despite this potential, the mechanism(s) of electrophile toxicity has not been fully elucidated. Our understanding of the electrophile-sensitive proteome also requires further refinement. In particular, it is important to know the physicochemical features of the electrophile/nucleophile reactants that shape the size and corresponding toxicological consequences of the inhibited proteome. In this regard, the responsive proteome might include electrophile sensitive proteins of mitochondrial origin, it should be realized, however, that this might not necessarily constitute specific targeting of this organelle. Also not well defined are the sequence of cytotoxic events that lead to cell injury and the intervening role of endogenous cytoprotective pathways (e.g., sirtuin 1 deacetylase enzyme, Keap1/Nrf2 pathway) in tempering this electrophile toxicity (see related discussions in Fritz and Petersen, 2013; Grimsrud et al., 2008; LoPachin and DeCaprio, 2005). Furthermore, it is of significant toxicological relevance to determine whether electrophiles can interact additively or synergistically. Thus, electrophiles from different sources might interact leading to acceleration of the disease processes or environmental toxicity. Defining mechanisms of electrophile toxicity is critical toward deciphering the relationship between electronic structure (soft, hard) and electrophile reactivity (electrophilicity). Such information represents a rational basis for predicting toxicity associated with new or unknown chemicals. A mechanistic understanding is also important for development of environmental remediation/avoidance strategies and for devising pharmacotherapeutic approaches to certain disease processes that involve environmental and/or endogenous electrophiles (e.g., see LoPachin et al., 2016).

Achieving an adequate level of mechanistic understanding however appears daunting when the diversity of electrophilic chemicals is presumed to reflect a diversity of corresponding molecular mechanisms. As presented in this Perspective, we have used calculated HSAB parameters ($\sigma$, $\eta$, $\omega$ and $\omega^{-}$) and knowledge of intervening physicochemical features to untangle the search for a rational electrophile mechanism (Fig. 1). Thus, we have realized that ACR, HNE, methylacrylate (MA) and acrolein are members of the same chemical family; i.e., $\alpha,\beta$-unsaturated carbonyl derivatives. HSAB-based calculations of softness ($\sigma$) and hardness ($\sigma$) indicate that these chemicals are soft electrophiles of variable electrophilicity ($\omega$; Table 1). According to HSAB principles, soft electrophiles preferentially form covalent adducts with the highly nucleophilic soft thiolate state of cysteine. In support of this theory, we and others have shown that ACR, HNE, MA and acrolein form Michael adducts with the thiolate state of cysteine-centered catalytic triads. Research has demonstrated that irreversible covalent reactions are responsible for protein inhibition and subsequent toxicity. We propose that our application of HSAB principles represents a rational approach toward deciphering molecular mechanisms of toxic electrophiles.

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## ABBREVIATIONS

| | |
|---|---|
| **HSAB** | hard and soft, acids and bases |
| **HIV** | human immunodeficiency virus |
| **DNA** | deoxyribonucleic acid |
| **RNA** | ribonucleic acid |
| **FMO** | frontier molecular orbital |
| **LUMO** | lowest unoccupied molecular orbital |
| **HOMO** | highest occupied molecular orbital |
| **Cys** | cysteine |
| **EA** | ethyl acrylate |
| **MMA** | methyl methacrylate |
| **$E_{LUMO}$** | LUMO energy |
| **PTP1B** | protein tyrosine phosphate 1B |
| **$LD_{50}$** | lethal oral dose for 50% of the population |
| **HNE** | 4-hydroxy-2-nonenal |
| **ONE** | 4-oxy-2-nonenal |
| **NAC** | *N*-acetylcysteine |
| **NSF** | N-ethylmaleimide sensitive factor |
| **SNAP-25** | Synaptosomal-associated protein of 25 kDa |
| **AGEs** | advanced glycation end products |
| **CMC** | S-(carbonxymethyl)cysteine |
| **SIRT3** | mitochondrial sirtuin3 |
| **GSTP1-1** | glutathione S-transferase P1-1 |
| **GAPDH** | glyceraldehydes 3-phosphate dehydrogenase |
| **NO** | nitric oxide |

| | |
|---|---|
| **$H_2O_2$** | hydrogen peroxide |
| **Nrf2/Keap1** | nuclear factor erythroid 2-related factor 2/kelch-like erythroid cell-derived protein with CNS homology-associated protein 1 |
| **eV** | electron volt |

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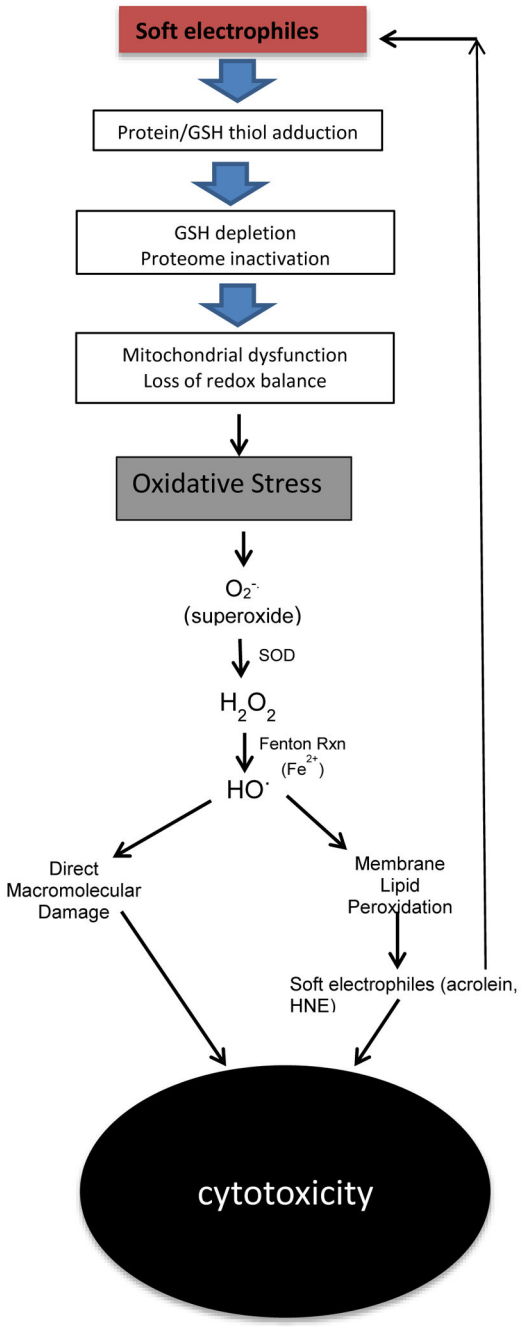


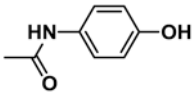
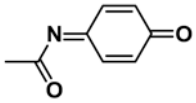
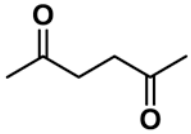
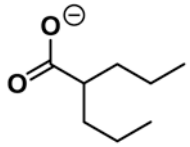
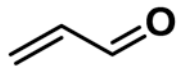
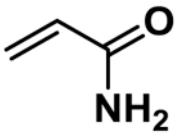
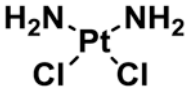

**Figure 1.**

This figure is a schematic representation of the major mechanistic aspects of a soft electrophile-initiated toxic cascade. Soft electrophiles preferentially form Michael adducts with soft nucleophilic thiolate residues. This promotes initial cellular GSH depletion and inactivation of proteins that are constituents of specific soft electrophile responsive proteomes. The resulting mitochondrial injury initiates cellular oxidative stress via generation of superoxide anions ($O2^{-}\cdot$) and hydrogen peroxide ($H_2O_2$). Through metal-catalyzed Fenton reactions, these free radicals generate highly reactive hydroxyl radicals ($OH^{-}\cdot$) that can cause direct macromolecular damage. In addition, the hydroxyl and

superoxide radicals can initiate peroxidation of membrane polyunsaturated fatty acids to yield α,β-unsaturated aldehydes (e.g., acrolein, 4-hydroxy-2-nonenal). As soft electrophiles, these endogenous aldehyde toxicants contribute to the cellular electrophile burden and can thereby augment cytotoxicity. Electrophile toxicity can be muted by activation of antioxidant cellular stress responses (e.g., sirtuin 1 deacetylase enzyme, Keap1/Nrf2 pathway). Hard-hard covalent interactions cause cytotoxicity via a common mechanism; i.e., disruption of discrete hard electrophile-sensitive cellular proteomes. Our recent research has shown that soft electrophile-mediated cascades (e.g., acrolein exposure; NAPQI intoxication) can be prevented by multifunctional enolate-forming compounds (e.g., 2', 4' 6'-trihydroxyacetophenone) that act as soft nucleophilic surrogate targets (LoPachin et al., 2016). Thus, since many pathogenic processes are mediated by soft electrophiles, soft nucleophilic scavengers could be highly effective cytoprotectants.

**Table 1.**

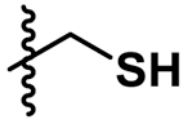
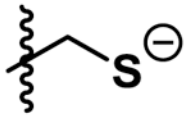
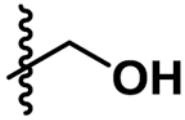
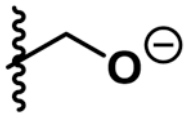
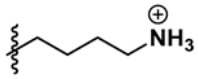
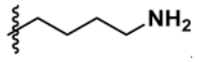
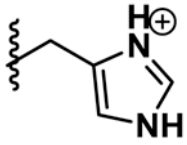
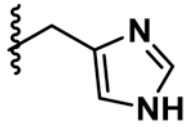
Calculated quantum mechanical parameters for selected electrophilic toxicants (parent compound or metabolite).[a]

| Compound | Structure | Softness ($\sigma$, $10^{-3}$ $eV^{-1}$) | Hardness ($\eta$, eV) | Electrophilicity ($\omega$, eV) |
|---|---|---|---|---|
| APAP |  | 217.86 | 4.59 | 0.92 |
| NAPQI (reactive metabolite of APAP) |  | 523.56 | 1.91 | 7.08 |
| n-Hexane (industial solvent) |  | 184.67 | 5.42 | 0.71 |
| 2,5-Hexanedione (metabolite of n-hexane) |  | 314.96 | 3.18 | 2.01 |
| Valproate (pharmaceutical) |  | 404.04 | 4.48 | 0.009 |
| Acrolein (industrial and endogeneous unsaturated aldehyde derivative |  | 371.75 | 2.69 | 3.57 |
| Acrylamide (food contaminant) |  | 327.33 | 3.06 | 2.52 |
| Cisplatin (platinum-based antipeoplastic) |  | 400 | 2.50 | 3.43 |

[a]Ground state equilibrium geometries were calculated for each structure with DF B3LYP-6-31G* in water from 6-31G* initial geometries. Values obtained were used to calculate $\sigma$ and $\omega$ (see text).

**Table 2.**

Calculated quantum mechanical parameters for selected nucleophilic targets.[a]

| Compound | Structure of R-group | Softness ($\sigma$, $eV^{-1}$) | Hardness ($\eta$, eV) | Nucleophilicity w/ Acrolein ($\omega^-$, eV) | Nucleophilicity w/ Chlorpyrifos ($\omega^-$, eV) |
|---|---|---|---|---|---|
| Cysteine |  | 1.724 | 0.58 | 0.0502 | 0.0421 |
| Cysteine anion |  | 0.601 | 1.67 | 0.6388 | 0.6097 |
| Serine |  | 0.289 | 3.46 | 0.0610 | 0.0485 |
| Serine anion |  | 0.373 | 2.68 | 0.9790 | 0.9414 |
| Lysine cation |  | 0.259 | 3.86 | 0.0632 | 0.0505 |
| Lysine |  | 0.296 | 3.38 | 0.0830 | 0.0684 |
| Histidine cation |  | 0.321 | 3.115 | 0.0092 | 0.0045 |
| Histidine |  | 0.298 | 3.36 | 0.1146 | 0.0976 |

[a]Ground state equilibrium geometries were calculated for each structure with DF B3LYP-6-31G* in water from 6-31G* initial geometries. Values obtained were used to calculate $\sigma$ and $\omega$ (see text).