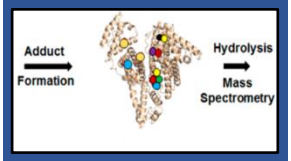
TOXGURUKUL FOUNDATION

TOXICOLOGY NEWSLETTERS



The capacity to blunder slightly is the real marvel of DNA. Without this special attribute, we would still be anaerobic bacteria and there would be no music.

-*Lewis Thomas*

June, 2020

Important Announcement

ToxGurukul Foundation Presents

ToxGurukul's Webinar Series

Resource person: **Dr. Wallace A. Hayes**
(Distinguished Fellow: American College of Toxicology; University of South Florida College of Public Health)

Topic: 'Fundamentals of Toxicology Part-II'

Date and Time: **July 11th, 2020, 8:00 p.m.** Indian Standard Time (IST). Duration: 90 minutes (1hr+30 min Q&A)

Course Moderator**: Dr. Varun Ahuja, (**M.V.Sc.-PhD, DABT)

Registration Link: Click Here

Please Note: We are also looking for **speakers for future webinars.** Speakers can be from anywhere from either in India or abroad.

Request for donations: Any sponsorship(s) to cover the cost of webinar conferencing**, please contact the undersigned (**drawn towards **Toxgurukul Foundation).**

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ADDUCTOMICS: AN INDISPENSABLE TOOL IN TOXICOLOGY

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INTRODUCTION:

Adductomics, at present, is making rapid leaps and bounds, and it is emerging as one of the significant tools in toxicological research. Adductomics added a new dimension of studying the toxic effects of the exogenous chemical agents and it is anticipated to bring massive changes in the toxicological landscape. The word adductomics refers to study and investigation of several types of adducts formed in the biological system by exogenous chemical agents. Generally, we study DNA, RNA and Protein adducts and they are formed by the reaction of the chemical agents with the biological molecule's DNA, RNA and Proteins respectively. In most instances, the chemical agents do not directly bind with the biological molecules to form adducts, being non-reactive in nature. So, they are first converted to reactive intermediaries (electrophiles) that attack the susceptible and vulnerable sites in the biological molecules to form adducts and this reaction is catalyzed by Cytochrome P450 systems in the body.

Reactive electrophiles by forming adduct with DNA, RNA and Protein damages them resulting in several deleterious health complications. Formation of adducts is associated with multitude of pathological conditions such as diabetes, autoimmune diseases, cancer, birth defects and cardio-vascular diseases and this makes adductomics indispensable to gain deeper insights in these diseases and develop therapies to counter them. Adductomics potential is evident from its practical applications across diverse domains. It plays a pivotal role in prognosis of cancer, environment health assessment, development of personalized medicine and toxicity studies of the exogenous chemical agents.

Rapid stride in the tools to identify and quantify adducts have transformed adductomics to one of the most sought-after disciplines in toxicology. Numerous tools that are currently being used to diagnose the adducts in the biological systems are 32P-Postlabeling, fluorescence, immunoassay, electro- chemical detection and Mass Spectroscopy (LC-MS, GC-MS, CE- MS). Even though much progress has been achieved, a lot needs to be done to achieve precision in identifying and quantifying adducts, and considerable research is being done in this direction to address the shortfalls and deficits. To

get a lucid understanding of adductomics, we have discussed different types of adducts in detail along with their applications.

DNA ADDUCTS

Humans are exposed to a myriad of environmental and dietary genotoxicants that are detrimental to the DNA. Many of these toxicants are pro-carcinogens i.e. un-reactive and serve as precursors to carcinogen, and they need to be transformed to reactive metabolites (electrophiles) that react with DNA to form DNA adducts. This transformation is facilitated endogenously by catalytic xenobiotic enzymes (Cytochrome P450 Systems), which are broadly categorized as Phase I and Phase II enzymes. Reactive electrophiles generated from genotoxicants covalently modify the DNA by several mechanisms namely arylamination, alkylation, bis-electrophile cross-link formation, adduction with reactive intermediates of lipid peroxides or free radicals. The underlying reason for formation of adducts with the nucleotide bases in the DNA is they possess sites that are susceptible to attack by electrophiles (Fig 1.)

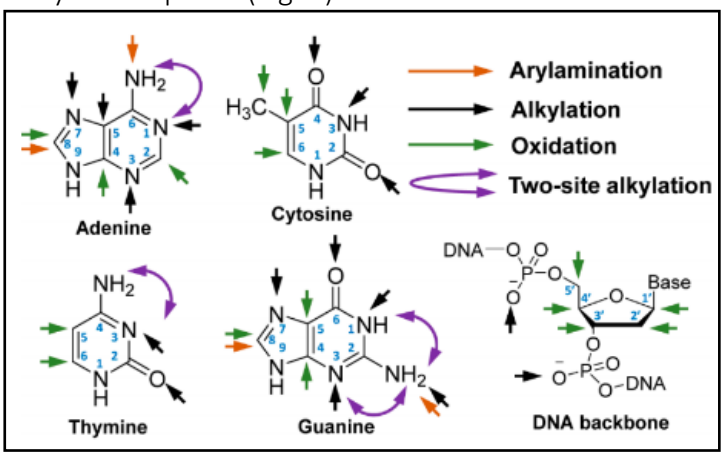
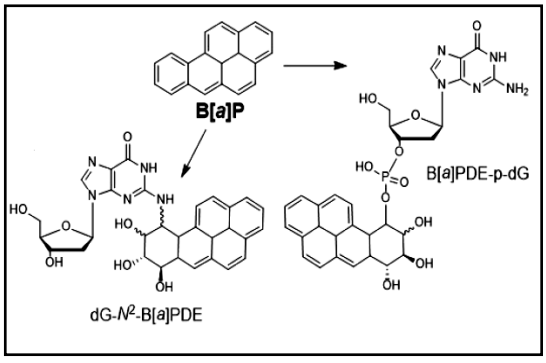


Figure 1: Reactive sites for adduction and oxidation in nucleotide bases.

The type and nature DNA adduct formed is dependent on multiple factors such as the chemical structure of the reactive chemicals, ability of the compounds to intercalate with DNA, and the nature of the electrophiles. This is can be substantiated by fact that aflatoxin, tobacco specific nitrosamine (NNK), polycyclic aromatic hydrocarbon (B[a]P), heterocyclic aromatic amine and other exogenous toxicants form different type of DNA adducts owing to their different structural and chemical properties.



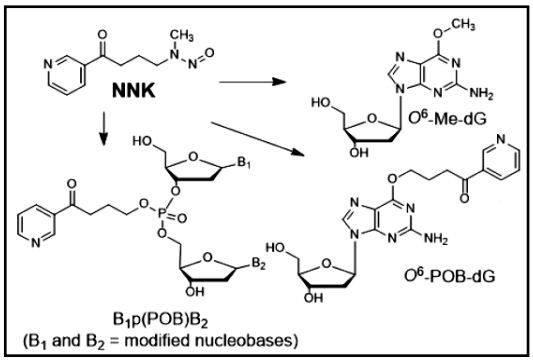


Figure 2: This figure shows DNA adducts formed by NNK, a tobacco specific nitrosamine and B[a]P, a reactive Polycyclic Aromatic Hydrocarbon (PAH). If we notice the structures of adducts, both the reactive electrophiles are reacting in a different fashion to generate different types of adducts.

Detrimental effects of the DNA adduct are birth defects and chronic diseases. Moreover, a study revealed that high prevalence and spike of several chronic diseases is due to formation of DNA adducts when exposed to environmental pollutants, and this unraveled new secret hidden behind the etiology. DNA adducts trigger carcinogenesis by inducing mutations and altering gene expression, and the effect is more predominant if the DNA adducts are formed at tumour related genes. Over a period, deeper insights in DNA adductomics revealed a strong correlation between amounts of DNA adduct and risk of carcinogenesis, paving the path for development of biomarkers. Several selective DNA adducts were also developed as potential biomarkers for forecasting and prediction of cancer, and this would facilitate to counter the disease and develop preventive health strategies. The measurement of DNA adducts formed by the potential carcinogens in target organs is one of the most direct methods to assess the genotoxic potential of a chemical compound, and this is infact a new strategy to evaluate the genotoxicity of a chemical.

DNA adducts are detected and quantified by several tools and the most predominantly used one is LC-MS, which generates a unique fragment pattern for DNA with and without adducts. Moreover, the characteristic feature of the fragmentation pattern of DNA adduct is near universal loss of the deoxy-ribose from the parent molecule, as a result we get peaks at $[M+H-116]^+$ (Fig 3).

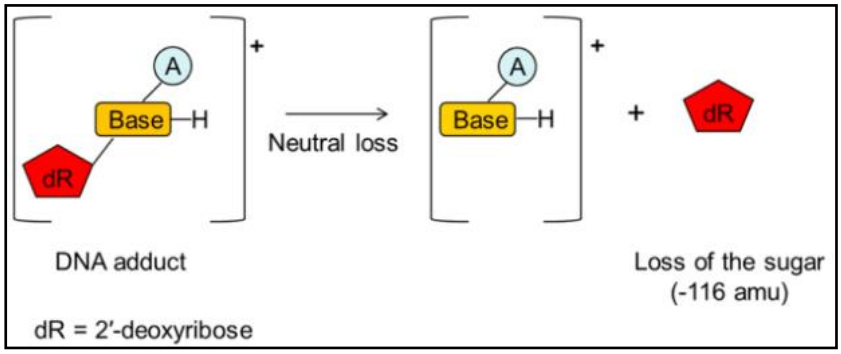


Figure 3: Neutral loss of deoxy sugar from nucleotides in DNA.

In the example below, how DNA adductomics can be analyzed by LC-MS is explained in detail. The HepG2 cells without and with exposure

to Benzo[a]pyrene generated different fragmentation patterns in mass spectroscopy and the DNA with adducts is distinguished from the other by putative adducts indicated by I, II and III. From the above putative adducts, adduct II was taken and results of LC-HRMS CID of adduct revealed five abundant diagnostic product ions at m/z 454.1499, m/z 303.1007, m/z 285.0902, m/z 257.0954, and m/z 152.0563. Detection of m/z 454.1499 provides us the confirmatory evidence for the loss of 2′-deoxyribose from the parent protonated molecule ([M + 2H – dR]$^+$) and corresponded to a loss of 116.0474 amu.

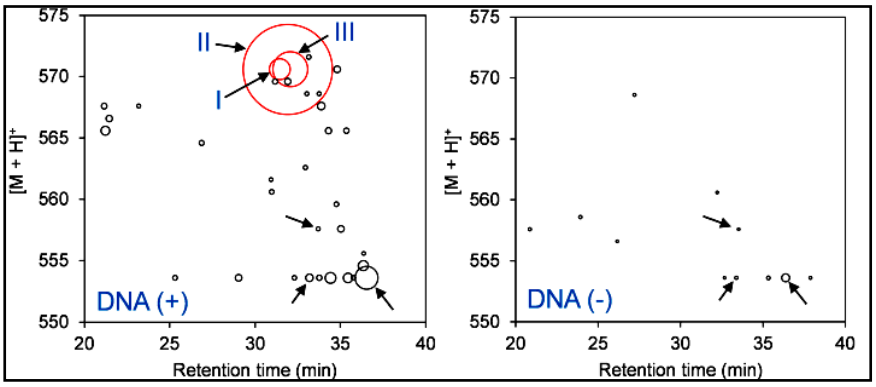


Figure 4: Results of the DNA adductomics analysis of the DNA isolated from HepG2 cells with and without exposure to Benzo[a]pyrene digested to 2-deoxynucleosides and analyzed by LC/ESI (+)-MS/MS utilizing SRM mode.

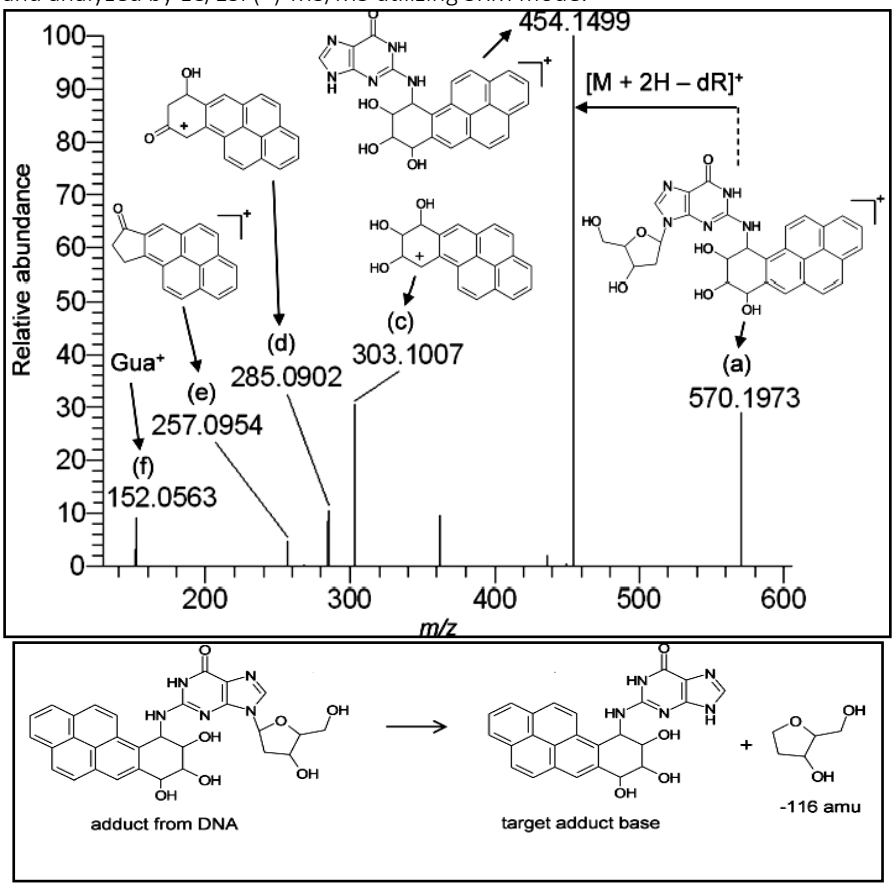


Figure 5: Results of LC-HRMS product ion scan analyses of putative DNA adduct II showing fragmentation pattern with characteristic M + 2H – dR]$^+$ at 454.1499 amu of protonated Guanine.

RNA ADDUCTS:

Similar to the DNA, RNA also forms adducts with reactive electrophiles to form RNA adducts as nucleotide bases of RNA also possess the vulnerable sites with which the electrophiles react covalently to form deleterious adducts. When compared with DNA adducts, RNA adducts is not extensively studies and we have few research publications in this regard. Similar to DNA adducts, cells with and without RNA adduct yield different fragmentation pattern in LC-MS, and characteristic feature of the fragmentation pattern of RNA adduct is near universal loss of the ribose, instead of deoxy-ribose sugar in DNA, from the parent molecule, as a result we get peaks at $[M+H-132]^{+\cdot}$

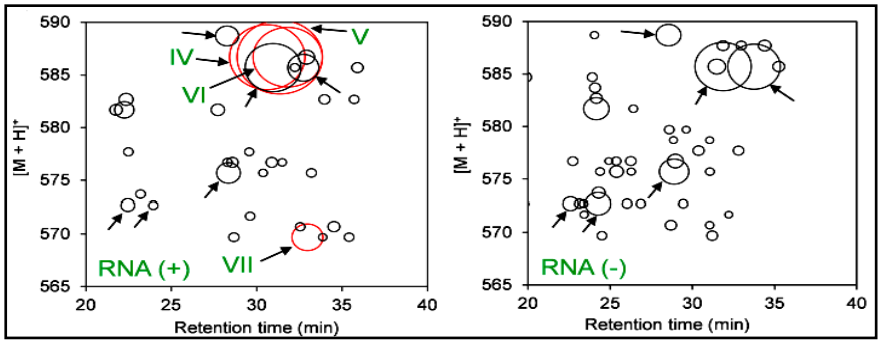


Figure 6: Results of the RNA adductomics analysis of the RNA isolated from HepG2 cells with and without exposure to Benzo[a]pyrene digested to 2-deoxynucleosides and analyzed by LC/ESI (+)-MS/MS utilizing SRM mode.

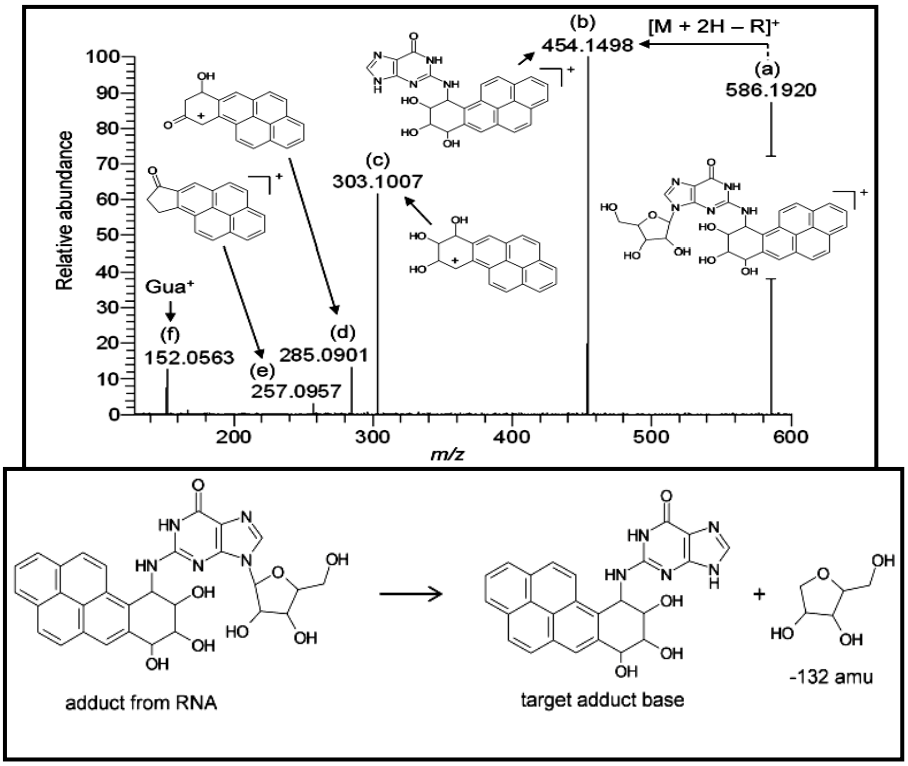


Figure 7: Results of LC-HRMS product ion scan analyses of putative RNA adduct V showing fragmentation pattern with characteristic M + 2H – dR]⁺ at 454.1499 amu of protonated Guanine.

RNA molecules play a vital role in several cellular mechanisms such as transcription and translation, which are key for protein synthesis. Formation of RNA adducts might result in damage to the RNA and impairs functions carried by it, triggering lethal health consequences.

PROTEIN ADDUCTS

While the role of DNA adducts in the chemical carcinogenesis dates back to the 1960s and 1970s, the evidence for protein adducts emerged in the late 1970s and 1980s. In the early 1970s, the covalent binding of drugs to proteins as a mechanism of toxicity emerged when studies were conducted with acetaminophen, using the 14C-radiolabeled molecule, which covalently binds to liver proteins. This gave evidence that reactive electrophilic intermediates, which are largely the products of Cytochrome P450 metabolism, damage proteins and can lead to toxicity. The precursors for the reactive electrophilic intermediate, to name a few, are pollutants, drugs, pesticides, herbicides, enals, isocyanates, arylamines, and aflatoxin. Similar to that of DNA, proteins also encompass chemically susceptible sites at which electrophiles react to form deleterious adducts. There are several proteins such as albumin, hemoglobin, glutathione, histones in the body which form adducts with reactive chemicals. Protein adductomics has several applications in toxicological research. A research study revealed that biomonitoring of protein-adducts of carcinogens is superior and an alternative to the measurement of DNA adducts for assessing exposure. In a recently published study regarding a chemical toxicant (furan, a most commonly present food contaminant) revealed that protein adduct formed by the reaction of electrophiles with histones would destabilize epigenetic regulation of genes, and this results in altered gene expression initiating prejudicial mutagenesis.

In another study albumin was selected, being the most abundant protein, which reacts with carcinogens and reactive metabolic intermediates originating from the drugs. Albumin forms adduct with the human carcinogens *c.f.* aflatoxin B1 and benzene, which were successfully exploited as biomarkers to address the role of these chemicals in cancer risk in molecular epidemiology studies. In addition, Albumin forms adducts with many therapeutic drugs or their reactive metabolites such as acetylsalicylic acid, β-lactam antibiotics, non-steroidal anti-inflammatory drugs, acetaminophen, chemotherapeutic agents, and antiretroviral therapy drugs. The identification and characterization of the adduct structures formed by Alb with drug metabolites are playing a key role to gain greater understanding of the generation of reactive metabolites and assisting to predict idiosyncratic drug reactions and toxicities. The reaction of drug metabolites with Alb is currently employed as part of the battery of screening tools to appraise the potential toxicities of drugs. This

example provides us with evidence that protein adducts aid in estimating the biologically effective dosage of drugs and developing personalized treatment approaches, thereby providing safe medication. Several protein-adducts are also becoming handy to predict early and late biological effects of several chemicals and toxicants.

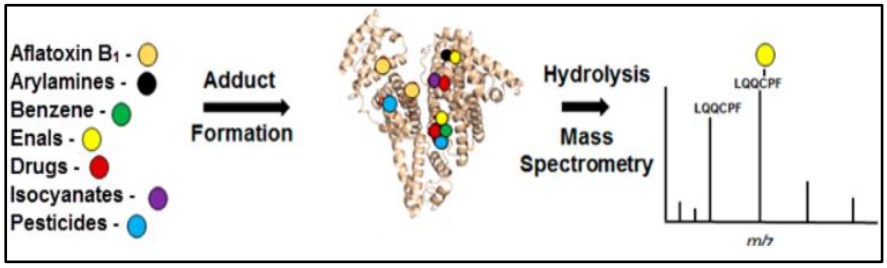


Figure 8: Protein adduct with reactive compounds and analysis their analysis by Mass Spectroscopy

Studying protein adducts also facilitate in evaluating the quality of environment as proteins in several biological systems easily form adducts with toxicants, and the extent and type of adduct provide us vital information pertinent to quantity and category of pollutants prevalent in the environment. This is substantiated by the fact that some of the common industrial pollutants such as benzene, isocyanates, naphthalene, aromatic amines form adducts with albumin. Now coming to the tools to detect and study protein adducts, several approaches are designed over a period and they are shown in the figure below.

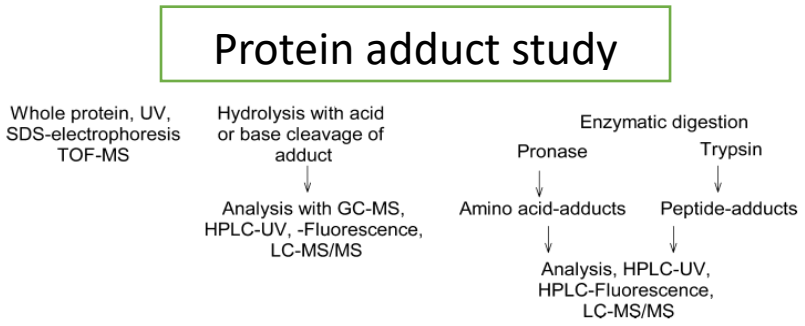


Figure 9: Protein adduct study tools and approaches.

From among the several tools used for identifying adducts, LC-MS is the most predominantly used one and to identify unknown adducts we generally compare the spectra data of unknown compounds with the reference adduct. Firstly, reference adducts should be synthesized by assuming a particular electrophile and later they should be compared with the unknown adducts of interest. Reference adducts can simply be generated by adding the proposed precursor electrophiles to either plasma or whole blood/lysate and they are subjected to fragmentation using LC-MS. The synthetic adducts should be then compared with the unidentified adducts with regard to m/z of the precursor ions, fragmentation patterns as well as

retention times, which is shown in the figure below. Moreover, as the time progresses, this approach also contributes to generate an extensive database of the reference protein adducts by which identification of unknown protein adducts becomes much easier in coming times.

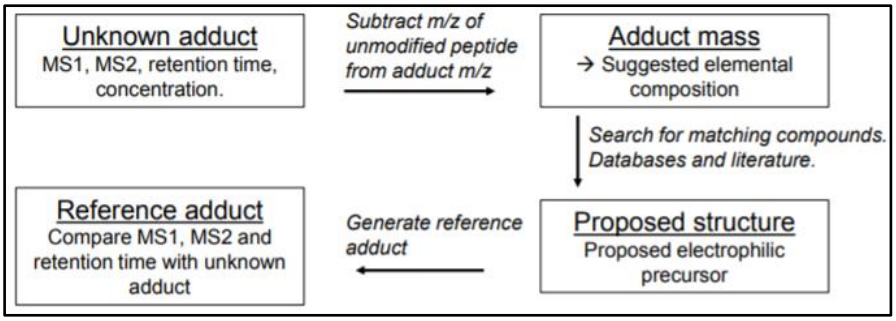


Figure 10: Flowchart showcasing the steps for identifying unknown adducts using adductome data using reference adduct.

CONCLUSION

Exposure to chemicals in the environment, diet, and endogenous electrophiles leads to chemical modification of several biological molecules and this damages them disrupting their functionality. Though rapid progress in technology made adductomics an indispensable role in toxicological research, it is not devoid of limitations and challenges. Targeted analysis of adducts helped in identifying different types of adducts but it would be more beneficial if all adducts of a particular category (DNA, RNA and Protein adduct) are identified simultaneously in a single assay. Why this is important because in real time scenario organisms are exposed to myriad of the chemicals and form multitude of adducts in chorus and assessing adducts all together provides us a holistic picture of toxicity in the biological systems and environment. By employing data-dependent acquisition and data-independent acquisition methods in the new screening technologies, high-resolution mass spectrometry (MS) instrumentation and non-targeted "omics'' have been playing a key role to screen simultaneously different types of adducts, but notable challenges in data processing must be overwhelmed to become a mature technology. In addition, adducts in humans are of low abundance, and this makes it relatively difficult for current softwares to reliably detect them when using common MS data acquisition methods. This provides the need for improvement to existing MS data processing softwares and creation of new algorithms for adduct detection to develop adductomics into a powerful tool for the identification of reactive electrophile agents. Furthermore, improvements in sample preparation and cleanup are also needed, along with improvement in sensitivity and selectivity. Despite of the fact that several hundreds and thousands of adducts have been characterized by the Mass Spectroscopy, very few attempts were made to create adductomics data base from the know data. This

should be addressed by creating a comprehensive database of all category of adducts so that identification and characterization of adducts become effortless, further assisting in the advancement of the field of adductomics.

The pace of technological advancements gives us optimism that limitations that are confronting the adductomics from reaching its fullest potential will be addresses soon, and time is impending fast for adductomics to reach zenith, equipping us with the new horizon in toxicological research.

QUESTIONS

1. What do you understand by Adductomics?
2. What is the difference between DNA, RNA and Protein Adductomics?
3. What is the relevance of Adductomics in Toxicological Research?

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ToxGurukul Foundation is a registered non-profit organization for professionals in the field of toxicology who are in search of a platform to learn and share the vast knowledge in this area. This syndicate belongs to independent professionals from different backgrounds of toxicology who share their knowledge to un-puzzle the Rubik's cube that each face in their daily work routine.

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