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The possible methods of diagnosis and effects of exposure to organophosphate pesticides

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Abstract. Organophosphate pesticide (OP), being one of those easy to access, has resulted in many cases of exposure specifically to those working field with it being commonly applied on a frequent basis. It is critical that those working with it have knowledge of its effects and the possible symptoms because of its exposure. And to those who may arrive without any relative detail about themselves, a possible method of diagnosis to confirm that the said person is at risk of exposure. Through investigation across multiple methods aiming to suggest possible pathways of obtaining detailed descriptions or further information of symptoms caused by OP exposure. Additionally, diagnosis methods and comparison to chemicals with similarity in property or classification of OP. In the aim to propose a viable method, comparison and suggestions for current applications.

Keywords: Organophosphate pesticides, chemical poisoning, diagnosis, symptoms, area of effects, organophosphates

1. Introduction

Organophosphate pesticides (OP), some of which are highly toxic, were first developed in Germany during the 1940s, soon becoming widely applied in use to defend against pests in agriculture [1]. Its major content is the product of the esterification between phosphoric acid and alcohol, which are the main component for herbicides and insecticides, and also, a nerve agent as nerve gas in chemical warfare [2]. Additionally, it has been used in public health applications [1], homes, gardens and veterinary practices. OP poisons animals, including insects, amphibians, birds and mammals. Thirty-six of them are registered in the United States, all of which could potentially result in OP poisoning. Exposure to OP will phosphorylate the acetylcholinesterase enzymes (AChE) at the nerve endings, resulting in not only a loss in the number of accessible AChE to the rest of the nervous network but also the overstimulation of the effector by the excess acetylcholine (ACh). Exposure can occur through inhalation [3], absorption by the skin after contamination [4], consumption of food products with the application of OP [2].

Solutions had been discovered for those under the effect of OP, with treatments aiming to reduce lethality and maintain stable condition of the patient. Before commencing treatment, the patient's contact with heavily contaminated items such as clothing and objects should be avoided. Protective gear and gloves should be supplied and worn for anyone treating the patient being affected by OP to prevent the spread of contamination of it. Atropine antidotes could be applied to the patient, aiming to antagonize the effects of excessive concentration of acetylcholine near the end of nerves to reduce

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risks brought about by the symptoms and damage to the nerve cells. However, atropine does not reactivate the cholinesterase enzyme nor catalyze the disposal of organophosphorus. As soon as it wears off, recrudescence of poisoning may occur resulting in multiple doses being required for stability of the condition. Then, the patient should be observed for at least 72 hours to ensure none of the symptoms reoccur [3]. Although those are not the only required procedures, applying them in the process of diagnosis and treatment prevents further damage caused to the body and reduces the risk of contamination to other individuals.

Depending on the length of exposure and method of absorption of OP, the symptoms that can be observed vary [2], some being significant in the immediate diagnosis of OP exposure. A wide range of effects can take place including the respiratory system, cardiovascular system, central nervous system, gastrointestinal system and renal system [2], with a few examples being anxiety, seizures, hallucinations, memory loss, and circulatory or respiratory depression. These effects are not transitory, but long-term effects lasting many years can occur [2,5].

More animal studies than human ones have been carried out to determine further effects of the symptoms caused by OP [6]. This is due to poor accessibility to suitable patients and the difficulties of following up and keeping records, as well as the challenges of carrying out investigations on the corresponding organs. Presenting the limitation for any further finding via observation on patients. A few methods in replacement are in vitro or in vivo, in response to the incapability of carrying out a fully functional study for a few organs. Suggesting that those with similar functionality and inner structure can be experimented upon to receive comparable results of what could possibly happen when OP exposure has been detected in humans. To develop matching responses and relative treatments, or to discover a reliable method for diagnosis of OP poisoning. In vivo studies on the effect of pesticides on the hemolysis of red blood cells sourced from albino rats showed that there was no link between lipid per oxidation and hemolysis itself and K+ leakage was found, which those effects could have possibly been the different stages throughout exposure to OP while the cell membranes become damaged, and those sites of action being initiated by it [7].

To diagnose the presence of OP poisoning typically, it is difficult to interpret its presence from symptoms directly, as most can be shared with other illnesses or chemicals. Though tests and results are still relatively applicable with a combination of the patient's own data record, even though being unable to be confirmatory simply based on produced results by far. Previously, measurement of blood cholinesterase was carried out involving measuring the level of plasma cholinesterase (ChE) and AChE inhibited by those absorbed OP to determine the influence. However, it has come to be realized that the said method is insensitive, as a result of the variation of individual cholinesterase activity which will require both post-exposure and baseline samples from the patient. Alternatively, another method is to analyze the number of metabolites in urine [4], currently the most sensitive out of all methods of measurement [8]. The analysis carries out on either those specifically being the metabolites of OP or dialkyl phosphate metabolites (DAPs) [4], which act as the biomarker for OP. As some have involvement in the detoxification of OP such as paraoxonase, its activity being modified by the oxidative stress caused by OP [8]. Recently, developments have occurred where OP exposure can be diagnosed through the extraction of butyrylcholinesterase(BChE) from human serum and measure the adducts after digested with pepsin [9]. Those methods will be analyzed further in the following paragraphs.

2. Effect of poisoning

2.1. Mechanism for organophosphate pesticide to take effect

In further detail of how OP reacts with the human body, the major effect of poisoning takes place in the nervous system, by having the ability to bind to AChE within the red blood cells [5], typically an amino acid on the enzyme eliminating the 'leaving group' [10]. AChE hydrolyzes ACh, a neurotransmitter transmitting nerve impulses from one nerve to another across the synapses. Therefore terminating the effect of it on the receptors that have wide coverage in the body [2], thus if AChE has

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been inactivated by organophosphate through the process of phosphorylation of the hydroxyl group on it [5], preventing the breakdown of ACh leading to overstimulation of nicotinic and muscarinic receptors [2]. With an overabundance amount of ACh being present in the synapses, this can potentially lead to fasciculations and myoclonic jerks, eventually resulting in flaccid paralysis because of the depolarizing block. Furthermore, hypertension, sweating, tachycardia and leukocytosis with a left shift can be observed. Varying for each compound, at some time the AChE-organophosphate compound will undergo aging which renders the enzyme to be resistant to reactivation, reducing or preventing some methods of treatment to be effective [5].

2.2. Categories of symptoms

The observation of the difference in symptoms allowed them to be classified into three criteria dependent on the length of exposure: Acute, which occurs from immediate exposure to 24 hours, subacute, occurring from a range of 24 hours to 2 weeks, and chronic, also known as long term effect, lasting beyond weeks up till years. As shown previously, OP poisoning is capable of affecting the respiratory system(aspiration pneumonia, progressive respiratory failure, severe bronchospasm, noncardiogenic pulmonary edema), cardiovascular system(arrhythmias, bradycardia, hypertension, hypotension, prolonged QTc), central nervous system(psychosis, seizure, mental status changes, hallucination), gastrointestinal and metabolic system(pancreatitis, hyperglycemia, low bicarbonate) and renal system(acute kidney injury) [2]. Additionally, intermediate neurologic symptoms which occurs within 24 to 96 hours after exposure [5]. Moreover, the rapidness of symptoms is dependent on the pathway of exposure, which the most rapid responses can be observed as a result of inhalation. By basing on the system affected complications of cases can be determined [2].

3. Investigating more possible responses

According to previous studies, there is a limited number of recorded data about exposure to OP due to most cases being located in rural areas with an extensive application of herbicides, insecticides and pesticides [2]. This raised difficulties in conducting investigations as the patient might be under exposure to multiple chemicals with a combination of symptoms, resulting in incapability of determining the actual effect in certain circumstances. Also, difficulty in access to such patients caused challenges for researchers to locate a suitable exposed patient to monitor. In return to resolve this in vivo and in vitro experimentation can be carried out as a replacement with the aim to observe and investigate based on its similar properties. For more in-depth detail of possible undiscovered symptoms and responses according to OP exposure varying in time and dosage. Further assisting for development in diagnosis of OP poisoning. Or computer simulation to simulate the condition of certain organs and their response to OP.

3.1. Replacement organs

Although replacement of human organs in learning their mechanism, responses and structure is a practical method, precisely, the difference between actual human organs and animal replacements is still present. Under real-time scenarios what happens may be more complicated due to the involvement of human-specific response mechanisms to the presence of xenobiotics. For instance, a commonly used replacement in the study of the human heart is sheep heart, as they are highly similar to each other, as well as the body weight and the cardiac output of both humans and sheep: the cardiovascular system of the sheep itself and the system for humans consisted of two circulations; the intracardiac pressure within sheep is approximately the same as human; the coronary veins have a huge similarity. In contrast, there are still noticeable differences: the position of the heart within a sheep is less significantly to the left, but more vertically tilted; a blunt apex next to the left ventricle; the body weight ratio is 3g/kg, whereas for human it is 5g/kg; the aortic valve have been described as more fragile and thin; the coronary circulation for sheep which the majority of its myocardium receives its blood supply from the left side [11].

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Another important human organ being studied mainly through similar replacement organs is the brain. In this case, ferrets are the clue offeror, being the model to study human brain development in the past 30 years. Compared to rodents, ferrets' brains have a significantly increased size; similar to human brains, they possess a gyrencephalic cortex and undergo extensive postnatal brain development. Moreover, ferret brain also has other features specific to human brain, such as outer sub-ventricular zone. Whilst similarities have been located in other mammals such as mice typically upon embryonic migration of interneurons to the cortex and postnatal ration of interneurons to the olfactory bulb, however the evidence is limited [12]. This leads to a brief summary that because of humans' own specialty, replacement organs could only assist in learning the structure and functionality of inner organisms, but they cannot fully replicate what will actually happen as slight differences may result in a significant change in what will be observed. Though, it is neither completely inaccurate, nor a waste of effort and time. However, some related experiments have contributed heavily to an impressive push-through in certain ongoing investigations in specific organs that are far greater than results achieved in past decades. In further detail, the results can be integrated with the aim of discovering not yet uncovered methods of diagnosis capable of distinguishing fully between multiple chemicals and their effects on the human body, avoiding confusion as much as possible.

3.2. Simulation

Regarding the recent developments and investments in technology, the increasing growth may be able to provide benefits or methods. Which is capable of simulating the response of certain organ from existing database, known knowledge and predict those yet unknown to human. From noted cases and records about the functionality of, for example, the heart. An outstanding combination of current technology and detailed understanding of the function and structure of a specific organism makes it completely possible to create a simulation program with the capability of simulating various scenarios. It can fill out the blanks and support what has always been a huge downside of research in relation to human response to exposure of poisonous xenobiotics, with the rare or nearly impossible cases to study and investigate. The growth of simulations provided an excellent pathway for scientists to convert organs into digital computer code. A given set of values being inputted to the program and formulas, to calculate and determine the relative effects. The model that has been reviewed upon is a combination of a few previous models before its foundation, consisting multiple angles to view and simulate the heart in the form of separate components. It has led to the current variation where all components are put altogether as a whole, being proficient at simulating both normal and diseased conditions. In aiming for maximum accuracy and to give the greatest detail possible, multiple elements are put in consideration. This includes the shape and size of the left ventricle, the motion of the muscle fibers, muscle thermodynamics, the structure, shape and size of human heart. Through calculations, a part of the heart itself can be represented by 3D shapes performing certain movement, applied with few assumptions of the condition and the complete structure of the simulation. According to existing studies about the movement of heart muscles, a phenomenological theory has been developed and applied to the data for this heart model. A set of equations (Voigt and Maxwell "models") were applied to calculate the force-length-velocity relation between the rate and the force driving the process of contraction. After comparison with other theories, within sufficient records the one that had been founded by Bornhorst and Minardi is implemented to present details.

Although by altering the numbers the contraction of all the sarcomeres can be governed, it only is applicable to fully stimulated myofilaments. To convert from unstimulated to fully stimulated condition, it is accommodated by a stimulation factor. Further experiments carried out in order to obtain more data to improve the simulation had shown that the contractile dynamics of the muscle fibers cannot be simply represented and described by the formula, as there has been no consideration of the elasticity of the fiber, which was noticed to play an important part in the contraction movement of the heart. Multiple approaches have been refined to combine both aspects of the muscles using conceptual mechanical analogies of the muscle fiber through spring models. With the financialized model, after entering the required input, with some limitation of the length of the simulation, the range

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of the input, what has been entered can be processed and then calculated to output the result. Although the accuracy of the simulation and illustration of the predictions on the time variation of several variables are still yet unmeasurable. Along with a few other factors that cannot be measured yet, which can be assumed according to expected values. Nevertheless, the accuracy and the detail this simulation can supply is indeed greatly aiding the process of acknowledgement of the human heart [13].

4. Diagnosis of organophosphate pesticides

Taking a thorough look at OP poisoning and its effect upon the human body as a whole, the structure of organophosphate containing also sulfur, nitrogen and oxygen, can be involved in multiple metabolic pathways due the involvement of multiple groups of enzymes which also metabolize other xenobiotics. This feature made OP the subject to both phase one and phase two metabolic reactions. The metabolic and chemical lability lead to it being unable to intact readily in neither the environment nor the organism, which this property contributes to its effects on the human nervous system takes place [14].

4.1. Current applied method

At present, there are two known approaches to determine the presence of OP, one of them being carrying out measurements of the level of ChE and AChE within human plasma. Both have been a practical method of OP exposure measurement and monitoring those working under considerable risk of exposure for several years. Yet, depression of the plasma ChE enzyme activity is not necessarily associated with the symptoms caused by anti-cholinergic toxicity, and large depression has been detected without the effect of OP taking place. Decrease in the red cell enzyme activity has been shown to have a closer relation to those symptoms. Hence, measurement of both enzymes will be suggested. Under the case of severe exposure up till the point that measuring the level of ChE and AChE leaves no ambiguity, extra information must be obtained in order to retrieve accurate interpretation. A wide range of inter-individual plasma ChE activity resulted in the average of population not applicable, which made it necessary to acquire the baseline value of said activity from the individual. There is no challenge in retrieving such data in a lab monitor but with accidental exposure the actual sample taking will be delayed approximately 60 days after.[4] It is a rapid and inexpensive method that is easy to conduct, as it provides useful information about the occurrence of exposure. For easy access, it has been made into a mobile kit for analyzing fresh samples [9]. The high reliance of this method on the normal enzyme activity in order to obtain the decrease in its level is the main issue, also what has been mentioned being the inter- and intra-individual variation in ChE values renders the severity of exposure[4]. This makes it difficult to interpret the condition of the patient without pre-exposure measurements, which generally are not available for most encountered patients [9].

Another method is through measuring the urinary metabolites, in contrast less invasive and logistically simpler [4]. A method applied to analyze the urine sample obtained from the patient is gas chromatography, which presence is confirmed using a chromatograph/mass spectrometer [15]. Another technique of analyzing the urine sample is by determining the color complex as a result of the reaction of OP and 4-(4-nitrobenzyl)pyridine(NBP). Which under the effect of OP the coloring complex produced maintained as a characteristic purplish blue for several hours [16]. Although, there are a few guidance values for urinary metabolite, it can be used to assess the efficiency control procedures and assist in reducing exposure [4]. Despite the fact that those metabolites have a relatively short half-life, resulting in difficulties determining the result several days after exposure. Additionally, the metabolites being measured can form naturally, which measurements tends to be an overestimation of the exposures presenting false positives [9].

Regarding the detection of organic molecules, the electrochemical activity of organophosphorus pesticides is an available method to analyze its poisoning effects. It has been recognized as highly sensitive, which currently improvements have been directed towards its sensitivity via modification of the electrode surface. Particularly, ZrO2 nanoparticles have demonstrated an excellent performance in detecting OP poisoning, as a selective sorbent for solid-state extraction, due to its affinity towards the

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phosphate group. In advantage, electrochemical analyzation is rapid, sensitive, selective and accurately, additionally, it is affordable, more portable as well which made it an uncomplicated way of detection for organophosphorus pesticide. However, it is not applicable for exposure detection [17].

4.2. Chemicals with similar properties

In response to the inadequate proficiency of present diagnosis methods of OP exposure, an applicable approach is to compare and contrast the mechanism of diagnosis. Especially those other pesticides with similar structure or area of effects, then substitute it into the current detection techniques to improve in affirmation of the result. Because of its chemical similarity, carbamates can be seen as a feasible option to venture upon to discover application of diagnosis that can be modified to become an alternative to the current methods [2]. Having equivalent area of effect as OP, carbamates cause carbamylation of AChE at neural synapses and neuromuscular junctions [18], with increased activity of AChE at the nicotinic and muscarinic receptors. The possible features shown at the central nervous system are not very prominent due to the poor permeability of carbamate across the blood-brain barrier [19]. Whilst the mechanism is similar, their process of binding is reversible. And its duration of toxicity is significantly less, typically below 24 hours, as a result of being hydrolyzed spontaneously. To conduct diagnosis on patients exposed to carbamates, there are three broad categories: ChE inhibitors, Cholinomimetics and Nicotine alkaloids [18]. Immediate measurement of enzymatic activity may be misleading because of the transient AChE effects of carbamates [19]. To obtain indications of carbamate poisoning, samples for blood pseudocholinesterase and red blood cell (RBC) AChE should be taken for examinations, unless an immense amount of N-methyl carbamate has been absorbed. If a sample has been taken in an hour or two it is unlikely to find the blood ChE levels being depressed. Even under the above circumstances an enzyme test must be applied as enzyme reactivation occurs in vitro and in vivo. The absorption of N-methyl carbamates can be confirmed by analysis of unique metabolites found in urine; alpha-nanophyl from carbaryl, isopropoxyphenol from propoxur, carbofuran phenol from carbofuran and aldicarb sulfone, sulfoxide and nitrile from aldicarb. Through complex analysis the responsible agents can be identified [20]. Clear indication shown from the close methods of diagnosis compared to organophosphates presented the possibility to invest further into the metabolites that are only specific to OP. However, due to the similarity between those for carbamates and organophosphates, it would not be capable in assisting the discovery of a confirmatory method of diagnosis.

Organophosphorus compounds all commonly contains carbon and phosphorus, although some are known for being an inhibitor of AChE and content of pesticides, many does not [10]. Specifically those ones capable of creating coordinating covalent bond between the sulfur and phosphor atoms [21]. Another use of organophosphate is being applied to materials used for furniture, textiles and building materials as organophosphate flame retardants (OPFR). Also, OPFR is often used as plasticizers in floor polishes, coatings, engineering thermoplastics and epoxy resins. It can be commonly detected within the atmosphere, dust, sediments, variety of soils, air, surface water, and a range of biological samples. Due to no chemical bonds are formed between OPFR and the product, it is relatively easy for it to escape into the environment. To detect its presence, especially within the environment being a common contamination site, a traditional method is through liquid chromatography-mass spectrometry (LC/MS). Samples extracted will be stored at approximately 2 to 8˚C until it returns to the ambient temperature for analysis to be conducted. This method avoids the time-consuming sample concentration or enrichment detection, at the same time allowed ease of operation, rapid detection and reliable results. With improvements in efficiency whilst eliminating other factors that may affect the outcome. Further developments are made based on LC/MS, including microwave assisted extraction of sample in combination with gel permeation [22].

4.3. Recent development of diagnosis

In comparison to methods such as RBC ChE level, which are likely to be more accurate in relation to the patient's condition. Particularly their personal information, as this could provide the xenobiotic

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that the patient has been exposed to in relation to the symptoms shown, acting as a double insurance for confirmation. In certain situations, the patients themselves are unaware or lack the capability to describe the situation clearly about the xenobiotic, or those who require a guardian or responsible relative with enough knowledge about the patient's condition. It is necessary to apply a diagnosis method which can determine and provide a dependable outcome. The latest progress made in developing a confirmatory report about OP exposure was in 2020, through extraction of BChE which is able to form adducts from OP compounds. To begin, protein G agarose bead spin columns were incubated and then washed with buffers at room temperature, in preparation for the following steps. Coated with antibodies, which were used to extract the adducted and unadducted BChE from human plasma, with analysis carried out before and after extraction by modified Ellman's assay. Chromatography was then carried out to obtain a separation gradient.

Parathion and dichlorvos are used as the model pesticides that are able to produce BChE-organophosphate adducts, both being commonly applied as pesticides as the adducts formed are identical to what several other pesticides would form. Furthermore, the diethoxyphospho and dimethoxyphospho forms can undergo additional hydrolysis causing the loss of one alkoxy group which turns the adducts into 'aged adducts'. Once the formation is complete, the binding of the organophosphate to BChE will be irreversible meaning that enzyme reactivation will no longer be effective.

The results obtained have shown that this newly developed method is sensitive and specific for classes of pesticides. The only requirement is centrifuging the extracted plasma to immunoseparate BChE for easy access; as the adducts formed have a long half-life of up to days, making it suitable for retrospective analysis of OP exposure. It has also increased in sensitivity and specificity after optimization of multiple parameters in association with the process of analysis, including the LC/MS (HPLC-MS-MS) method for additionally better resolution. The accuracy and precision of all calibrators were determined through the validation process of seven analytical runs, with an outcome of a range of values possible for reliable results. The adducts formed vary dependent on the length of exposure, allowing patients to be distinguished based on the outcome. Because of its freeze-thaw ability, this allowed samples to be stored in a frozen state for up to 4 days as no statistical difference was spotted between freshly measured peptide concentration and those stored at -20˚C or -80˚C. With a temperature of 4˚C and 22˚C, this allowed it to be kept up till 8 days, however, the functional activity reduced by 40% when stored at 4˚C.

The total preparation time for the diagnosis to carry out is 22 hours, after removing the required time for the production of protein G agarose beads the time requirement can be reduced by 18 hours. This means the whole procedure can be carried out completely below 5 hours. The high affinity of the protein beads enables it to be ideal for extractions of proteins from plasma, showing its capability to capture a high percentage of anti-BChE antibiotics without suffering from interferences caused by plasma albumin. The method itself remained simple, with centrifugation applied to extract the sample and what has been obtained is easily transferrable to standard laboratories. And utilization of Protein G-coated agarose beads provided solid support to immunoprecipitated BChE from plasma [9].

5. Conclusion

In summary, since the development and application of organophosphate in different fields, particularly focused on organophosphate pesticides after the end of chemical warfare and the ban on the use of nerve agents in most circumstances, much research has been carried out. This has led to a clean and detailed understanding with an in-depth description of the relative symptoms caused by AChE inhibition, via multiple pathways of exposure, including inhalation, consumption, and absorption. A number of symptoms observed, however, is commonly shared among unrelated diseases or other pesticides, which created a complex situation to determine the presence of OP simply based on observation. One approach to resolve this issue is to carry out further examination and observation until OP poisoning-specific symptoms are discovered. To some extent, this would be the simplest but the most time-consuming. This is on account of the fact that most patients being affected by OP have

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other factors that act in combination. Another obstacle comes from the small number of accessible patients, reducing the possibility of real-time monitoring, reducing the reliability of the results. However, using in vivo or in vitro tests as a replacement, more accurate results are obtained about OP and its effects. On the other hand, the developing technology is surely an applicable tool that can and has the capacity to assist in those situations. Simulations of organs so far separately, and in future collectively to simulate the possibilities through calculations of formulas by combining the inputs and transforming them into virtual imagery and live responses to changes.

Shifting focus to diagnosis methods, the current methods in practice are unable to produce confirmatory results either due to the range of baseline activity that cannot be determined simply through obtaining average within the area, or because the molecule has a short half-life, decreasing the accuracy of results after a short period of time. Patients with signalizing symptoms present themselves too late for the urine metabolite examination to be carried out. This leads to a greater reliance on information from and about the patient themselves, some with the pre-exposure baseline activity of ChE. To address a suitable optimized diagnosis method for confirmation of OP poisoning, chemicals with either similar area of effect, or chemicals under the same classification of organophosphate were researched upon. No possible substitution was found according to the obtained material about diagnosis of carbamate poisoning; in contrast, progress was made from environmental detection method of the presence of OPFR. From various examples it has been shown that analyzing the presence of chemicals, typically organophosphates, are relatively more effective and efficient through LC/MS. By modification of the target and method of extraction of the samples, it is possible to develop an applicable method that is comparable, reliable and fast.

Later down the line, a recent development was came across using this method with the extraction of organophosphate adducts formed by exposure. In comparison to present methods of diagnosis, this is much more reliable, effective and to some, cost efficient. There appear to be no confounding factors that might increase the difficulties to confirm exposure; much less time is required for the product to be produced, with further reduction from the pre-manufacturing of agarose beads; it can be performed at a low cost with easy transformation of sample to other laboratory. All those factors resulted in it being the apparent preferred method to diagnose the presence of OP. Yet, there has been no information about the actual application of this method in the field, meaning that it is likely still under development. Furthermore, organophosphates are not the only inhibitor of BChE, which might lead to additional investigations of the other inhibitors, and to determine a method to distinguish the product of those from the organophosphate adducts if confusion may be caused by them. Moreover, as the foundation of the process was published back in the end of the year 2020, it would be beneficial to carry out more related experimentation to further determine its limitations, the cost of all equipment involved, as well as to ensure that there is maximum accessibility to the public.

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